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## ERRATA AND AUTHORS' EMENDATIONS

Page 455, sixth line from bottom, insert after "second" the words "kernel of the "

Page 510, paragraph 3, line 4, change "its" to "their."

Page 524, line 3,  $\frac{d\phi}{dt_1}\theta$  should be  $\frac{d\phi}{dt_1}\theta$ ; line 5,  $\frac{d\rho}{dA}$  should be  $\frac{d\phi}{dA}$ ; line 6,  $\frac{d\phi}{dK} - (t-t_1)A$  should be  $\frac{d\phi}{dK} - (t-t_1)A$ .

line 8,  $V = \frac{2.302585 \cdot 10^K (t-t_1)}{[1+10^K (t-t_1)^2]}$  should be  $V = \frac{2.302585 \cdot 10^K (t-t_1)}{[1+10^K (t-t_1)^2]}$ ; line 10,  $\frac{d\rho}{dK}$  should be  $\frac{d\phi}{dK}$ ; line 18, "are given

in Tables 5 and 6," should be "are given in Tables 3 and 4."

Page 549, line 10, "A<sub>5</sub>" should be "A<sub>6</sub>", line 13, "R" should be "K "

Page 576, ninth line from bottom, insert word "strictly" between "not" and "legitimate."

Page 651, line 1, the exponent "9" should be "4 "

Page 770, last sentence in paragraph 3 should be at the end of paragraph 1.

Page 774, third line from bottom should precede fourth line from bottom.

Page 999, third line from bottom should read ". . . northwest, southwest, and southeast . . . "

Page 1021, insert "21" between "18" and "24" in the heading of Table 3.



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No. 1

## PRODUCTION OF FERTILE HYBRIDS IN THE ASCOMYCETE NEUROSPORA<sup>1</sup>

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### INTRODUCTION

The fact that monosporous mycelia of certain species of fungi in each of the large groups produce "perfect stages" only when mated with their reciprocal haplonts is now well established, and this type of segregation is referred to as heterothallism. In recent years various attempts have been made to produce hybrids by crossing different races, varieties, and species of fungi. Saito and Naganiski (8)<sup>2</sup> report the formation of zygospores in crosses of different forms of the Mucoraceae. Vandendries (10) obtained clamp connections in cultures where a strain of *Panaeolus campanulatus* was grown with a strain of *P. fimicola*. No fruit bodies were formed. Zattler (12) crossed two varieties of *Schizophyllum commune* and also two varieties of *Collybia velutipes*. The results, the author thinks, are proof of sexuality in the forms crossed. Burgeff (3) obtained zygospores when he crossed *Phycomyces nitens* with *P. blakesleeanus*, but the zygospores did not develop mycelia. Kniep (7) has secured conjugations between conidia, and more recently Dickinson (4) and Bauch (2) report fusions between germ tubes from sporidia of different species or races of smuts. These results are all highly interesting and valuable contributions to the genetics of fungi. The development of hybrid zygospores such as have been reported, or fusions between conidia or between hyphae of species of these groups of fungi, have not, it seems, so far resulted in the production of haplont spores or mycelia of a new generation.

In reporting on the culture work done in connection with their studies on heterothallism and homothallism in the genus *Neurospora*, Shear and Dodge (9) state that ascocarps were also secured by growing reciprocal haplonts of *N. sitophila* and *N. crassa* together, and by similarly mating *N. tetrasperma* with each of these two species. The manner of development of the fruit bodies and the characters of the ascocarps and their ascospores were such as to suggest that the ascocarps were probably true hybrid structures. The writer has continued the culture work with the three different hybrids. The results so far obtained by crossing the two eight-spored species, *N.*

<sup>1</sup> Received for publication Dec 1, 1927; issued February, 1928.

<sup>2</sup> Reference is made by number (*italic*) to Literature cited, p. 14.

*sitophila* and *N. crassa*, with the four-spored species, *N. tetrasperma*, illustrate very strikingly some of the principles involved in hybridizing ascomycetes.

## MATERIALS

### MORPHOLOGICAL FEATURES OF SPECIES CROSSED

Species of *Neurospora* furnish excellent material for genetical studies because of the ease with which true hybrid perithecia can be produced. The ascospores of these hybrids can be made to germinate and the mycelia in turn be made to give new generations of ascospores. The writer is referring here to hybrids between distinct species and not to crosses between mere races or varieties of the same species. The original descriptions (9) of the three species of *Neurospora* bring out the diagnostic features. *N. crassa* and *N. sitophila* are most nearly alike in their conidial condition. The ascospores are, however, very readily distinguished, and their ascocarps are unlike in size, color, and other characteristics. The perithecia of the former are larger and more nearly black than are those of the latter. These two species have been crossed and the ascospores from the fruit bodies which matured have been germinated. About 60 haplont mycelia so obtained have been grown in various combinations, with some highly interesting results. It is desired to report here, however, principally on the work done with *N. sitophila* and *N. tetrasperma* because of the more striking specific differences between these two species.

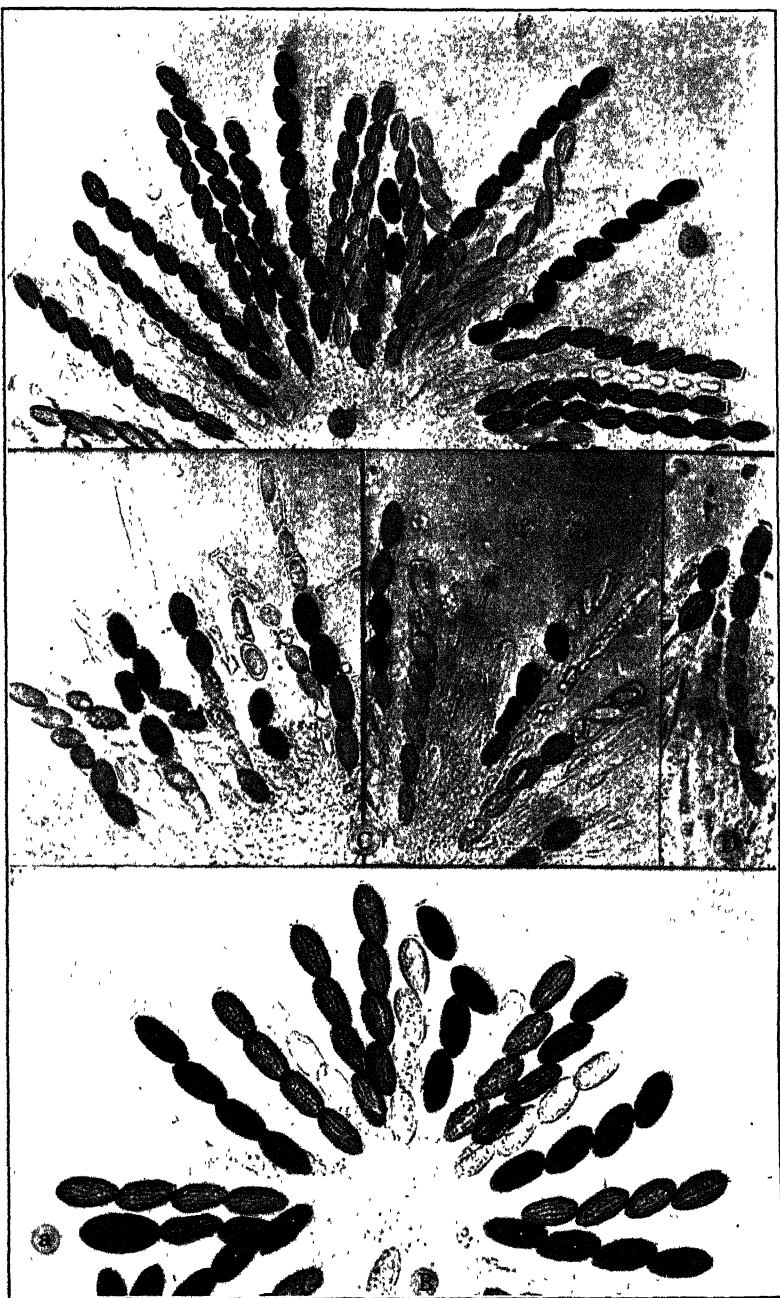
### CYTOLOGICAL FEATURES

The writer has recently (5) described nuclear behavior in the ascus of *Neurospora tetrasperma*, showing that each of the four spores originally includes two nuclei of opposite "sex." Furthermore, any spore which contains only one nucleus at the time it is cut out is much smaller than are normal spores, and it is unisexual. A haplont mycelium from such a small spore must be mated in culture with one of reciprocal sexuality in order to obtain perithecia. In other words, the normal spores of this species are homothallic or bisexual, and the small spores are unisexual or heterothallic. In the paper referred to above it was shown that segregation of the sex factors could take place in any one of the three nuclear divisions in the ascus and still result in the formation of bisexual spores. It seems probable, however, that segregation normally takes place in the first division. Wilcox (11) has determined culturally that in *N. sitophila* the spores in each half of an ascus alternate in pairs, two of one sex and two of another. Interpreting the results of her culture work in the light of what is found regarding nuclear behavior in the ascus, it is pointed out that here segregation of the sex factors must regularly take place in the second division of the ascus.

### EXPLANATORY LEGEND FOR PLATE 1

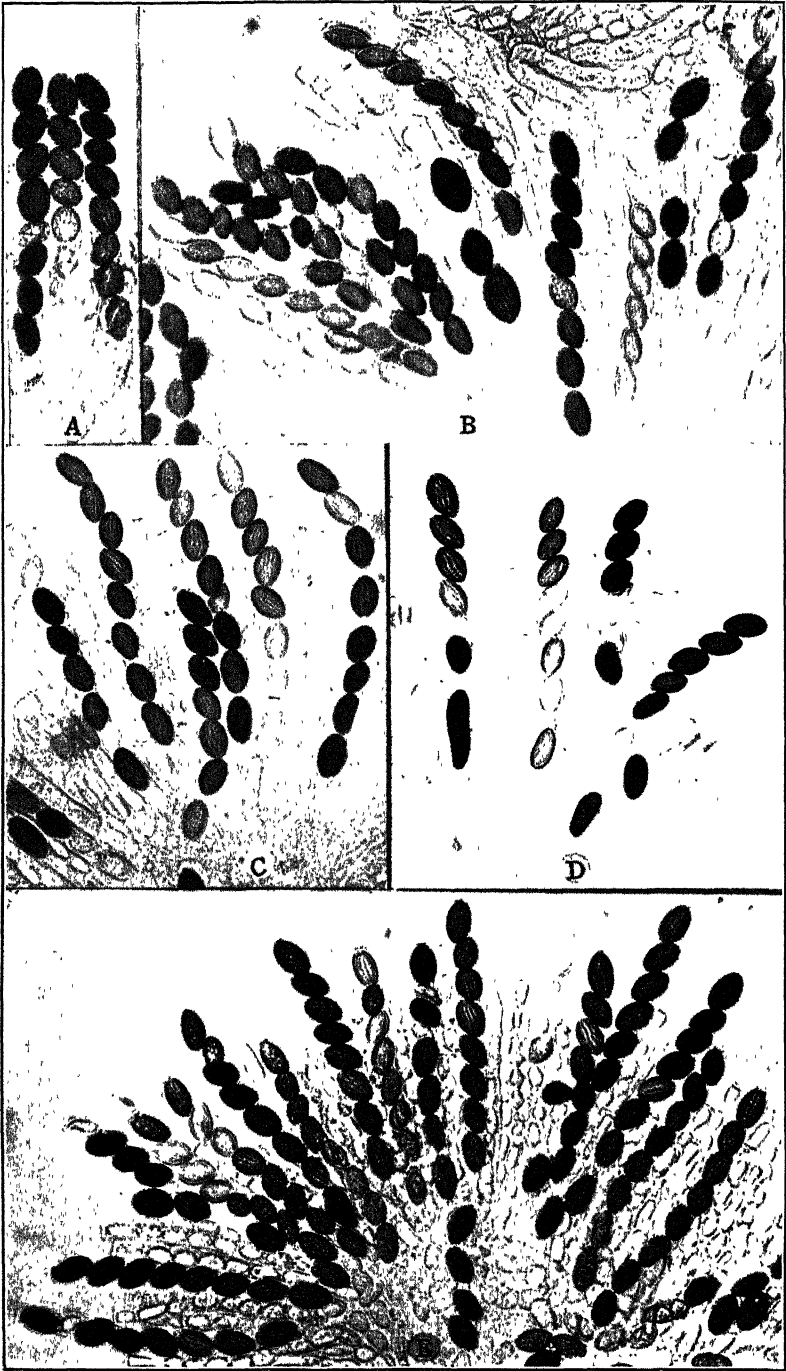
- A.—*Neurospora sitophila*; p<sub>1</sub> ascospores, Arlington strain. Asci regularly 8 spored. Only one ascus, a, with mature spores. The younger spores show the anastomosing striations more distinctly.  $\times 315$   
 B.—*Neurospora tetrasperma*; p<sub>2</sub> ascospores. All asci 4 spored except one, a, which has 1 large spore and 2 of normal size. Anastomosing ridges on the spores not as distinct as they are in the preceding species.  $\times 315$   
 C and D.—First-generation (f<sub>1</sub>) hybrid ascospores. Asci from a hybrid perithecium obtained by crossing *Neurospora sitophila* and *N. tetrasperma*. All asci shown here have 8 spores; many spores aborted after delimitation. Masses of mucilaginous material and partly disorganized hyphae among the asci mature ascospores varying somewhat in size. Rarely only 6 or 7 ascospores are delimited in such hybrid perithecia.  $\times 315$





Asci of *Neurospora sitophila*, A; *N. tetrasperma*, B; and *sitophila*  $\times$  *tetrasperma*, C, D.

For explanatory legend see page 2



Asci from second and third generations  
For explanatory legend see page 3

The cytology of the cross *Neurospora tetrasperma*  $\times$  *N. sitophila*, and particularly nuclear behavior in the asci in perithecia resulting from back crosses, is also proving highly interesting. The results so far obtained go to show most emphatically the necessity for cytological study as a basis for a correct interpretation of the results of culture work.

#### COMPARISON OF CHARACTERISTICS OF SPECIES CROSSED

Some of the characteristics of the two species of *Neurospora* crossed are given below in parallel and perhaps in order of their importance and reliability as marks of identification. Certain of these features are brought out in the works referred to (5, 9, 11) and in the illustrations included in this paper. Plate 1 shows asci and spores of *Neurospora sitophila*, *N. tetrasperma*, and of the first-generation cross. Plates 2, 3, and 4 show asci and spores of different generations of hybrids.

##### *Neurospora sitophila*

1. Heterothallic.
2. Asci eight spored.
3. Segregation of sex factors in the second division.
4. Ascospores at first uninucleate, at maturity binucleate.
5. Ascocarps begin to discharge spores within two or three weeks; fully mature in about three or four weeks.
6. Ascospores mostly 20–25  $\mu$  long.
7. Ascospores rather strongly ribbed.
8. Conidia bright salmon pink, profuse on corn-meal agar.
9. Perithecia 250–350  $\mu$  in diameter.
10. Asci long and narrow.

##### *Neurospora tetrasperma*

1. Homothallic.
2. Asci four spored.
3. Segregation of sex factors probably in first division.
4. Ascospores at first binucleate, at maturity four nucleate.
5. Ascocarps begin to discharge spores in about 10 days; fully mature in two weeks.
6. Ascospores mostly 30–33  $\mu$  long.
7. Ascospores faintly ribbed.
8. Conidia pale salmon pink to whitish, very sparse on corn-meal agar.
9. Perithecia 200–275  $\mu$  in diameter.
10. Asci rather short.

There is of course some variability in the features noted above. For example, when an ascus of *Neurospora tetrasperma* matures five or six spores, the spores will be of two different sizes, and the smallest spores will then be uninucleate and unisexual (5). Mycelia from these small spores develop scarcely any conidia on corn-meal agar. As regards the size of the perithecia, there is likewise some overlapping in the measurements in particular cases, and for each species the fruit bodies vary in size somewhat according to environmental conditions and according to the number produced in the culture. More numerous measurements show that the dimensions given by Shear and Dodge (9) do not indicate the differences in size accurately, but the figures in their Plate 4, A and B, show that the perithecium of

#### EXPLANATORY LEGEND FOR PLATE 2

A, B, and C.—Second-generation (f<sub>2</sub>) hybrid ascospores; asci from perithecia obtained by mating mycelia Nos. 8 and 9, derived from f<sub>1</sub> ascospores (pl. 1, C); perithecium developed as the result of crossing *Neurospora sitophila* and *N. tetrasperma*. Note some unevenness in maturity of the spores. Asci all 8 spored except the one shown at the center in B. This ascus originally delimited 5 spores, only 3 of which are approaching maturity; the 2 in the upper end are still hyaline. Counting from above, the 8 original nuclei are probably distributed as follows: 2, 1, 2, 1, 2.  $\times$  315

D.—Third-generation (f<sub>3</sub>) hybrid ascospores; asci from a perithecium obtained by mating Nos. 273 and 274, derived from f<sub>2</sub> ascospores similar in origin to those shown in A, B, and C. One ascus, at the left, delimited only 7 spores, one of which is much larger and probably contained 2 nuclei at its origin.  $\times$  315

E.—Third-generation (f<sub>3</sub>) hybrid ascospores; asci from a perithecium obtained by mating f<sub>2</sub> mycelia Nos. 256 and 233 derived from spores of an origin similar to Nos. 273 and 274. All asci 8 spored. Morphologically such perithecia would not be distinguished from perithecia of *Neurospora sitophila*.  $\times$  315

*N. tetrasperma* is only about three-fourths to four-fifths the size of the perithecium of *N. sitophila*. Measurements of 50 perithecia of each species at about the same stage of maturity indicate that this is about the average ratio of their sizes. With an abundance of material for study one usually has no difficulty in distinguishing the two species even if he considers only one of the diagnostic features noted above.

#### METHODS

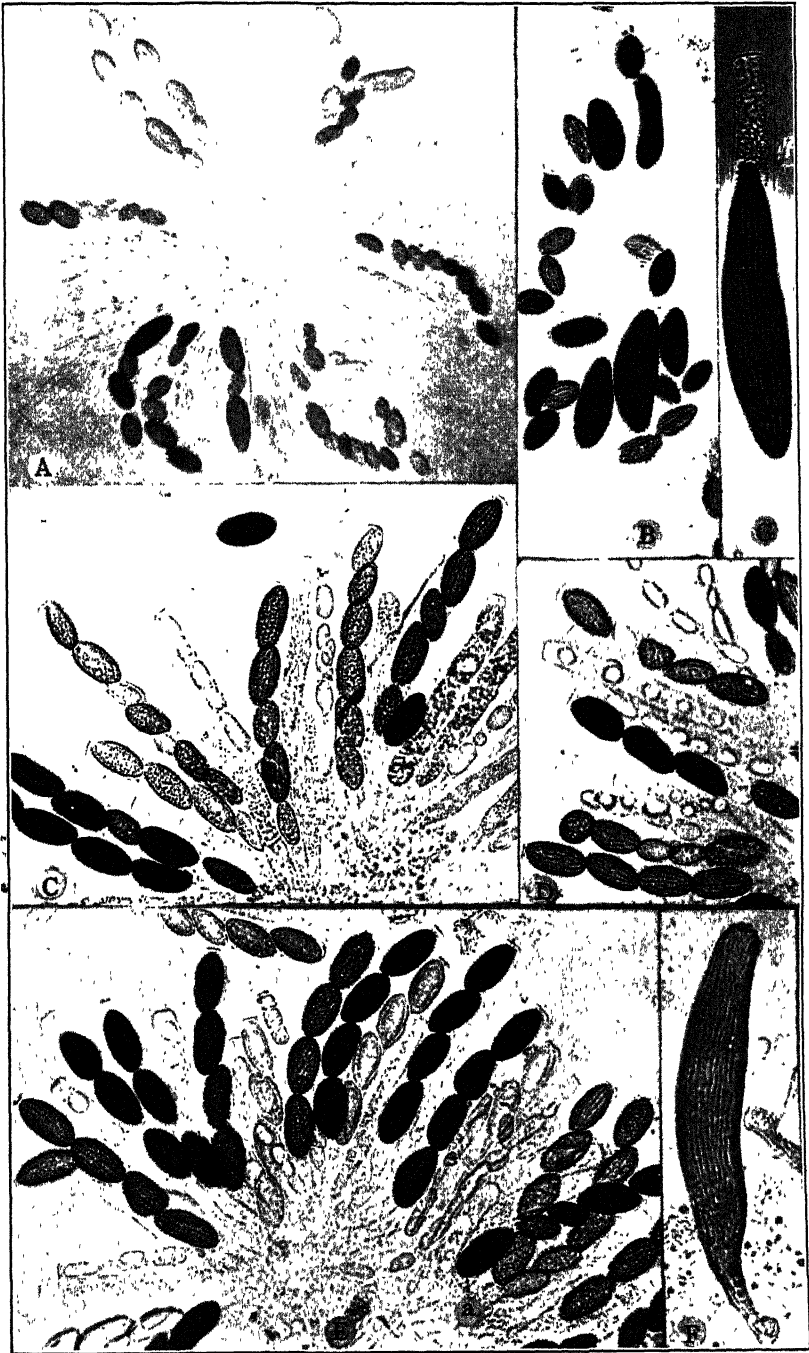
The method of inducing certain types of ascospores to germinate by heating them to 60 or 70° C. or more for a few minutes was originally worked out by the writer in connection with his studies on the Ascombolaceae. Additional special methods for culturing the *Neurospora* forms have also been rather fully described (9) and the precautions which are so necessary in this work have been emphasized. In case one is to work with cultures to be derived from ascospores from hybrid perithecia, the importance of excluding conidia is apparent. When haplont A of one species is grown with a reciprocal haplont B of the other parent species, and perithecia mature, the culture tube contains conidia of both parents in addition to ascospores from the hybrid fruit bodies.

The process by which the homothallic species *Neurospora tetrasperma* can be crossed with the heterothallic species *N. sitophila* will be described again briefly. In any spore print of the former species one can find a few small spores which measure from 20 to 25  $\mu$  or thereabout in length. As a rule, such small spores of this species are unisexual, as already noted. Of a number of small spores measured and later proved to be unisexual, the smallest was 18 by 11  $\mu$  and the largest was 29 by 12.5  $\mu$ . The average of 23 spores was 24.3 by 11  $\mu$ . The average size of the bisexual spores is about 31 by 14–15  $\mu$ . By growing the mycelia derived from the unisexual spores in various combinations, one can readily determine which are haplonts A and which haplonts B (9).

When any haplont A of *Neurospora tetrasperma*, for example, is grown with a haplont B of *N. sitophila* on corn-meal agar, there occurs a reaction which results at about the end of one week in the development of perithecial primordia scattered over the surface of the agar slant. While only a very few of these structures finally

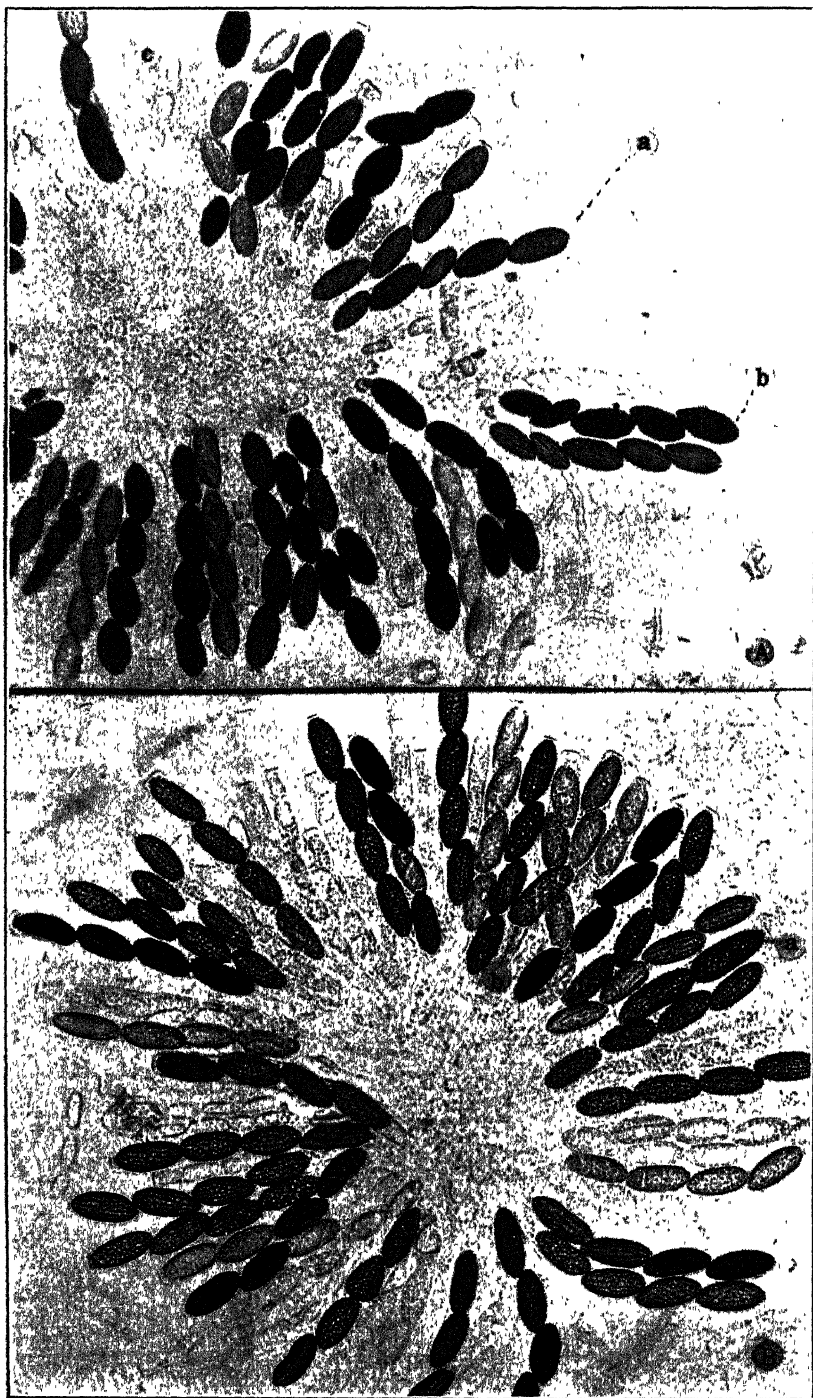
#### EXPLANATORY LEGEND FOR PLATE 3

- A.—First-generation back-cross ascospores; perithecium obtained by mating the mycelium  $S_2$  (haplont A) of the parent species *Neurospora tetrasperma* with the first-generation (f<sub>1</sub>) hybrid mycelium No. 5, derived from an ascospore from the cross *N. sitophila*  $\times$  *N. tetrasperma*. Asc with variable numbers of spores, from 3 to 8. No abortion of spores, the small spores containing only one nucleus at their origin and the larger spores more than one.  $\times 180$
- B.—Spores from a spore print of another back cross of similar origin except that  $S_1$  (haplont B) was mated with the f<sub>1</sub> mycelium No. 1. The largest spore shown is 57  $\mu$  long; the shortest is only 18  $\mu$  long. Such spores germinate readily when properly heated.  $\times 315$
- C.—Second-generation back-cross ascospores. Asc with from 4 to 7 spores. Perithecia obtained by mating mycelium  $S_1$ , a haplont B of *Neurospora tetrasperma*, with mycelium No. 207, derived from a small ascospore of an origin similar to those shown in A. Most of the asci from this perithecium have more than 4 spores.  $\times 315$
- D.—Another second-generation back cross resulting from similar mating,  $S_1 \times 208$ . Some asci in these perithecia have 4 spores, others have 5. There is some irregularity as to shape and size in the 5-spored type.  $\times 315$
- E.—Asci from another perithecium matured in the same culture as those shown in D. All asci here are 4 spored except two 5-spored asci shown at a.  $\times 315$
- F.—Olive-brown indurated ascus showing anastomosing ridges similar to those usually found on the walls of ascospores; no spores develop in such asci. The cause for the formation of these aborted asci has not been determined. Compare with G, a single-spored ascus.  $\times 570$
- G.—One-spored ascus of a back-cross hybrid. The end of the ascus shows above, the lower end not visible in the illustration. Compare with F.  $\times 570$



Asci from first and second back-cross generations

For explanatory legend see page 4



Asci of *Neurospora tetrasperma*, A, and of a second generation back cross, B

For explanatory legend see page 5

mature ascospores, they are young perithecia and essentially different from the sclerotiumlike bodies which so often develop in vast numbers in certain monosporous cultures.

As soon as small brown or blackish bodies begin to appear one has no difficulty in distinguishing which cultures contain reciprocal haplonts. These hybrid perithecia frequently fail to mature the ostiolar structure, but when a short beak with an ostiole is finally formed it may be taken to indicate that at least a few ascospores will eventually mature. It is on account of the slowness with which these structures mature that the writer is not as yet ready to furnish an adequate description of the first-generation perithecia. The first essential seemed to be the accumulation of mature  $f_1$  ascospores for further work.

### DESIGNATION OF MYCELIA AND HYBRID STRUCTURES

Mycelia of species of *Neurospora* produce coiled structures which, very likely, are morphologically equivalent to sex organs. The writer has made no attempt as yet to determine which haplonts are male and which are female. Sexual reproduction in these forms is a problem in itself which should receive careful study before definite statements are made in regard to the sex of particular haplonts. The two mycelia which are opposite in their reaction in the production of perithecia have been classed provisionally as haplonts A and B (9). Where symbols are helpful in the discussion, the system adopted by Allen (1, p. 558) may be used. The ancestral parents, *Neurospora sitophila* and *N. tetrasperma*, will be represented by  $p_1$  and  $p_2$ . The Columbia University strain of the former species being a haplont A, and the  $S_1$  strain of the latter species a haplont B, the first cross would be represented by  $p_1A \times p_2B$ . The  $F_1$  diploid generation consists of ascogenous hyphae and the young uninucleate asci. The  $f_1$  generation, begun with the completion of reduction divisions in the ascus, is represented by ascospores, their mycelia and conidia. Where a first-generation hybrid mycelium is mated with a haplont mycelium of the original parent, it is referred to in the usual way as a back cross and the resulting ascospores as first-generation back-cross spores, second-generation back-cross spores, etc., or, perhaps better, simply as spores from the first back cross, second back cross, etc.

### FIRST-GENERATION HYBRID STRUCTURES

#### PERITHECIA

The hybrid perithecia are essentially like those of the *sitophila* parent, averaging about the same size or a little larger. At the end of about one month the first ascospores can be found. Crushed mounts show a great irregularity in the size and state of maturity of spores in an ascus. As the perithecia are allowed to grow further it is clear that many asci contain eight spores, some hyaline and

#### EXPLANATORY LEGEND FOR PLATE 4

A.—Asci from a perithecium of the  $p_2$  parent, *Neurospora tetrasperma*. At *a* and *b* are shown 3 asci which have 5 spores each; at *c* an ascus with only 3 spores. Compare with asci containing second-generation back-cross ascospores, shown in B.  $\times 315$

B.—Asci from a perithecium obtained by mating a mycelium  $S_1$  with mycelium No. 209, which has an origin similar to mycelia Nos. 207-208 (see explanation of pl. 3, C and D). All asci 4 spored except one, *a*, which has 2 large spores. Another large loose spore, shown at *b*, may have belonged in a 3-spored ascus. The perithecia in this culture can not be distinguished morphologically from perithecia of the original  $p_1$  parent, *Neurospora sitophila*. Compare with A.  $\times 315$

only partly developed, others olive green or brown or black. (Pl. 1, C and D.) Sections also show that eight spores are commonly delimited. There can be no doubt that occasionally only six or seven are cut out and that the largest ones originally contain more than one nucleus. Very few asci of this hybrid mature the full complement of eight spores. This, no doubt, is due, however, to the abortion of certain spores after their delimitation.

#### MYCELIA FROM FIRST-GENERATION HYBRID ASCOSPORES

The particular culture from which the first-generation ( $f_1$ ) ascospores of the cross *sitophila*  $\times$  *tetrasperma* were obtained for further work was derived by mating the Columbia University (Cu) strain, which is a haplont A strain of *Neurospora sitophila*, with a monosporous unisexual mycelium  $S_1$  of *N. tetrasperma*, which is a haplont B (cf. Shear and Dodge (9, Table 6)). This culture or cross may be referred to simply as  $p_1 \times p_2$ . The culture appeared to have matured only a single perithecialium at the end of three months. It was on this account that it was chosen to furnish the desired ascospores. Some spores were obtained from the little spore print on the side of the test tube opposite the perithecialium, and others were gathered by crushing the fruit body. The ascospores were floated out on the surface of agar in a Petri dish, and some hours later they were heated sufficiently to kill the conidia while at the same time stimulating the ascospores to germinate. Sixty-two germinating ascospores which could be removed separately were originally transferred to agar in other Petri dishes, where 54 of them were measured. They were found to vary somewhat in size, the smallest being 18 by 11  $\mu$ , the largest 30 by 15  $\mu$ . The average, however, for the 54 spores measured was only 23.1 by 12.6  $\mu$ , which is practically the average size of the ascospores of *N. sitophila*. Normal ascospores of *N. tetrasperma* are larger where the average length of 60 spores judged to have been formed in 4-spored asci was 31.6  $\mu$ . After examination had shown that only a single ascospore had been selected in each case, the young mycelium was transferred to corn-meal agar tubes. None of the cultures except No. 34 has ever produced perithecia when grown alone. This one fertile culture was probably a mixture resulting from a contamination by accidental transfer of conidia which had not been killed during the heating process. The ascospore transferred measured only 22 by 8  $\mu$ , which is too small for a bisexual spore. In any event, the nature of this mycelium can be determined at any time by plating out single conidia, and it need not be considered further at this time.

#### FIRST-GENERATION HYBRID CONIDIA

Haplont mycelia of *Neurospora sitophila*, from whatever source obtained, agree in one particular, and that is in the production of an abundance of salmon-pink conidia. Individual strains, however, differ greatly in the number of sterile bulbils or sclerotiumlike bodies which are developed in culture. The Bainier and also the Berkhout strains (9, p. 1029) produce large numbers of these small bodies in corn-meal agar cultures. Some strains also tend to darken the culture medium. On the other hand, strains like B 704 and the Rose and the Cooley (9, p. 1029) develop no bulbils or bodies other than conidia,



and the corn-meal agar cultures are not darkened. All of the uni-sexual mycelia of *N. tetrasperma* (9, p. 1036) produce the sterile blackish bodies in great numbers. Some of the bodies are so large as to be easily mistaken for young perithecia. Very few conidia are developed in corn-meal agar cultures. Fertile cultures of this species from bisexual spores invariably develop large numbers of perithecia and some pale salmon-pink conidia, but only a very few of the sterile bodies. The corn-meal agar is not darkened. These facts are mentioned here because of their bearing on the selection and identification of hybrid strains.

The original  $p_1 \times p_2$  culture in which perithecia developed, giving the first generation hybrid  $f_1$  ascospores, of course contains conidia of each parent species. If, however, the factor for conidia production should be segregated out during the reduction divisions in the ascus at the end of the  $F_1$  generation, then monosporous mycelia derived from  $f_1$  ascospores should show differences in the characteristics of the  $f_1$  conidia formed. The growth of conidia in the  $f_1$  cultures was therefore watched with interest. Within a few days most of the cultures developed large numbers of conidia of the *sitophila* parent type. Not a single culture showed the very scanty production of conidia characteristic of *tetrasperma*. In cultures Nos. 1, 4, 5, 13, 19, and perhaps some others, the conidia are somewhat paler, and fewer are produced, suggesting a large element of *tetrasperma* inheritance. As the cultures age, still greater differences are noticeable, particularly with regard to the darkening of the culture medium. These differences seem to be rather constant, as shown in the three sets of sub-cultures made by transfer of conidia from the original cultures. The following mycelia develop an abundance of the salmon-pink type of conidia without any darkening of the agar: Nos. 2, 8, 9, 12, 14, 17, 22, 23, 25, 27, 35, 40, 41, 44, 45, 49, 56, 58, 62. Other cultures which produce the *sitophila* type of conidia, but which darken the medium and tend to produce many dark-colored sterile bodies, are the following: Nos. 3, 6, 7, 10, 11, 14, 15, 18, 31, 32, 33, 35, 36, 43, 53, 54, 55, 59, 61. These differences with regard to the production of conidia, bulbils, and the darkening of the medium just noted, are possibly no greater than would be found in case an equal number of haplont mycelia of *Neurospora sitophila* were gathered from different parts of the world and grown under similar conditions. No great care was taken to secure a standardized corn-meal agar medium or to place the cultures in exactly the same environment, so that the abundance of conidia produced in any cases reported in this paper is merely suggestive.

#### SEXUALITY OF FIRST-GENERATION HYBRID MYCELIA

The sexual nature of the 60 first-generation ( $f_1$ ) hybrid mycelia has been determined as compared with the reciprocal haplonts A and B of *Neurospora sitophila* and  $S_6$  and  $S_1$  of *N. tetrasperma*. This was done by pairing in culture each mycelium with haplonts  $S_6$  and  $S_1$ . The pairing could also have been made with haplonts A and B of the other parent, *N. sitophila*, and this was done in a number of cases, but the reactions are somewhat slower and perhaps less positive when this is done. In most cases the reactions are strong enough to indicate within a week the sexuality of haplonts grown against  $S_6$  and  $S_1$ . Perithecia develop so slowly in certain combinations as to delay

positive determination. It is to be expected that, wholly aside from the question of heterothallism, certain combination cultures would produce more perithecia than others.

The first-generation ( $f_1$ ) hybrid mycelia which have been classed as haplonts A include Nos. 1, 2, 4, 6, 8, 11, 12, 13, (14), 16, 17, 19, 20, 22, 23, 24, 25, 26, 29, 30, 31, 35, 36, 40, 41, 42, 47, 49, 50, 51, 52, 54, 56, 57, 60, 63. Those  $f_1$  mycelia which were found to be of the opposite sex, haplonts B, are Nos. 3, 5, 7, 9, 10, 15, 18, 21, 27, 32, 33, 37, 38, 39, 43, 44, 45, 46, 53, 55, 58, 59, 61, 62. The first-generation ( $f_1$ ) hybrid ascospores were originally selected at random from among those which germinated, yet 36 mycelia are haplonts A and 24 are haplonts B. The spore from which No. 14 was derived was not measured, but it was known to be a large spore. Two other cultures, Nos. 100 and 101, isolated later, were also from large spores, 33  $\mu$  and 29  $\mu$  long, respectively, and it was expected that all three spores would prove to be bisexual. They were, however, found to be unisexual and reacted like haplonts A. This would suggest that while only a single nucleus may have been included in the spores in the ascus, some of the spores failed to mature, so that the additional food was available for an increase in size of certain other spores which did mature. On one occasion two spores of *Neurospora tetrasperma*, 33  $\mu$  and 29.5  $\mu$  long, were proved to be unisexual, and each mycelium corresponds sexually to haplonts A ( $S_0$ ) of this species. Ordinarily spores of *N. tetrasperma* of such a size would be bisexual. The three conditions which may account for great irregularity in the size of spores in an ascus have been discussed recently by the writer (6). No doubt bisexual first-generation ( $f_1$ ) hybrid ascospores are occasionally developed, but from the results noted above it is clear that the hybrid structures resulting from crossing of *N. sitophila* and *N. tetrasperma* tend to resemble the *sitophila* parent in the production of unisexual spores as well as in the type of conidia produced.

#### RECOVERY OF PARENT TYPES

Haplonts Nos. 1 to 10 of the first-generation hybrid mycelia were grown together in pairs in all possible combinations, to determine whether any combination would result in the development of a pure *sitophila* or *tetrasperma* plant. Here also certain combination cultures produce an abundance of salmon-pink conidia while other combinations develop only a few of the paler type of conidia. Mycelia Nos. 1 and 5, when mated, superficially resemble cultures of *tetrasperma*, and mycelia Nos. 8 and 9 mated in culture resemble the *sitophila* parent. In the type of asci and ascospores produced, a corresponding correlated resemblance was apparent. Details will be given later, but it may be stated here that a great many asci in the perithecia, formed in the cultures where mycelia Nos. 1 and 5 were mated with their reciprocal haplonts of *tetrasperma*, develop large spores with from 2 to 5 spores in each ascus. Very rarely are 8 spores of equal size formed. On the other hand, many asci in the culture in which mycelia Nos. 8 and 9 are mated tend to develop quite regularly 8 spores. Asci which originally delimited fewer than 8 spores have also been found. The back-cross cultures in which either mycelium No. 1 or No. 5 was mated with  $p_2$  were particularly selected as a basis for the recovery of the *tetrasperma* parent, and cultures in

which Nos. 8 and 9 were mated together were chosen to furnish spores for the purpose of recovering the *sitophila* parent. Further study may show that the other haplonts might have been selected with more satisfactory results.

#### RECOVERY OF SITOPHILA TYPE BY REPEATED SELFING

As previously noted, both mycelia Nos. 8 and 9 derived from first-generation hybrid ascospores show an abundance of conidia of the *sitophila* type. In culture 8 the old conidia are somewhat paler and the agar medium finally becomes slightly darkened. These haplonts are of opposite sex and produce perithecia abundantly when grown together. The asci are long and slender, and 8 spores are usually delimited. (Pl. 2, A and C.) Very rarely, however, one finds an ascus with only 6 or 7 spores. In such an event the result is reflected in the difference in size of the spores in the ascus. The spores containing more than one nucleus at their origin will be considerably larger. At the center in Plate 2, B, is shown an ascus with 5 spores. Two large spores and 1 small one are mature. Another large spore can be seen in the upper end of the ascus. Just below this is a small spore with a large oil globule; both are still hyaline. Each of the 3 large spores probably contains 2 nuclei at its origin, and the 2 small spores only 1 each. Since haplonts 8 and 9 together produce perithecia in which most of the asci are 8 spored, the suggestion is strong that such a segregation may have taken place that haplonts of the pure *sitophila* parent could be selected out at once. Against such an assumption, however, was the evidence presented when the  $f_1$  mycelia were backcrossed with mycelia  $S_1$  and  $S_2$  of the *tetrasperma* ( $p_2$ ) parent. In no case were perithecia formed in the cultures in a way corresponding to the results obtained when the two parents,  $p_1$  and  $p_2$ , were originally crossed.

A sowing of the second-generation ( $f_2$ ) hybrid ascospores (pl. 2, C) resulting from the combination  $8+9$  ( $f_1A \times f_1B$ ) was made. Of the spores which germinated in such a position as to be easily isolated, 58 were measured and their mycelia grown in the usual way. There appears to be but little variation in the size of the spores chosen, their average size being  $20.2 \mu$  by  $13.4 \mu$ . Comparing the spores of the  $p_1$  *sitophila* parent (pl. 1, A) with these second-generation ( $f_2$ ) hybrid spores (pl. 2, A to C), it will be seen that if anything the  $f_2$  spores tend to be a little shorter or plumper. It should be noted, however, that the measurements given are of mature germinated spores, while the figures show mostly rather immature spores. Superficially, the second-generation ( $f_2$ ) hybrid mycelia Nos. 220 to 277 look much alike except that certain cultures, Nos. 223, 243, 264, 269, 273, 274, and 276, seem to produce fewer conidia which are paler in mass. Mycelia Nos. 224, 229, 234, 245, and 265 are intermediate with respect to the abundance and height of color of the conidia under the conditions in which they have been grown. The other cultures produce an abundance of conidia. The 58 mycelia all proved to be unisexual, and by growing them separately with the reciprocal haplonts  $S_2$  and  $S_1$  of the *tetrasperma* ( $p_2$ ) parent it has been found that 41 are sexually like haplonts A and 17 are like haplonts B previously referred to.

Growing the mycelia which produce paler and fewer conidia together in pairs shows that some trace of the *Neurospora tetrasperma*

parentage is still to be found in some of the  $f_3$  spores. One occasionally finds asci with six or seven spores, certain ones of which will be larger. (Pl. 2, D.) Under the same cultural conditions, the pure *sitophila* parent would show a remarkable uniformity of size and state of maturity of spores in an ascus. (Pl. 1, A.)

Those mycelia of the second ( $f_2$ ) generation which are of opposite sex and which produce conidia most like *Neurospora sitophila* have also been grown together in pairs, with the result that certain combinations produce perithecia with asci and ascospores (pl. 2, E) that would not ordinarily be distinguished morphologically from *N. sitophila*. The asci produced in the culture combination 256 + 233 are all eight spored.

#### RECOVERY OF THE TETRASPERMA TYPE BY REPEATED BACK CROSSING

The first-generation hybrid mycelia Nos. 1 and 5 which produce fewer and paler conidia, as noted previously, and which are of opposite sex were mated in a back-cross combination with haplonts  $S_1$  and  $S_6$  of the *tetrasperma* ( $p_2$ ) parent. Perithecia develop abundantly within about 10 days. When mycelium No. 1 is back crossed the asci formed tend to produce one or two very large spores (pl. 3, B) with one or more small spores in the same ascus. One of the perithecia examined showed a majority of the asci with one or two giant spores. The largest spore shown in this figure was 57  $\mu$  long. The largest spore found in these back-cross generations measured 62 by 25  $\mu$ . Such a large spore was germinated, and its mycelium produced perithecia a majority of whose asci had more than four spores. Cultures from giant spores seem to show no special tendency toward the development of asci with large spores.

Asci in the perithecia resulting from the back-cross combination where mycelium No. 5 was used likewise show a variability in the number, size, time of ripening and markings of the spores. (Pl. 3, A.) Individual perithecia also vary in the types of asci matured. In one case, which is in some contrast with the one shown in this figure, the numbers of spores delimited in the asci present at the time were as follows: 5, 7, 6, 6, 8, 4, 6, 5, 6, 5, 7, 5, 4, 5, 7, 8, 6, 7. The spores in one eight-spored ascus varied in size as follows: 27.5 by 14.5  $\mu$ , 26 by 14.5  $\mu$ , 22 by 13  $\mu$ , 20 by 13  $\mu$ , 25.5 by 14.4  $\mu$ , 25.5 by 14.4  $\mu$ , 20 by 13  $\mu$ , and 24 by 13  $\mu$ .

Monosporous back-cross mycelia Nos. 200 to 210, inclusive, were derived from selections of spores maturing in the culture in which the  $f_1$  mycelium No. 5 was mated or back crossed with mycelium  $S_6$  of the *tetrasperma* ( $p_2$ ) parent. It so happened that of all of the spores of various sizes sowed at this time the only ones which germinated and which were so situated as to be readily isolated were comparatively small, ranging in size from about 20 to 27  $\mu$  long. These spores were not actually measured. Contrary to what was expected, these mycelia produce scarcely any conidia, and no perithecia were obtained in the 121 cultures representing all of their possible combinations in pairs, duplicating each combination. When, however, the 11 mycelia were paired in back-cross combinations, it was clear from the results that none of them had lost its sexuality. No perithecia develop in cultures where they are mated with mycelium  $S_6$ , yet when they are mated with mycelium  $S_1$ , perithecia are produced abundantly, show-

ing that all of these 11 second-generation back-cross mycelia are of one sex and correspond to haplonts A.

Some months later another sowing was made of the first back-cross ascospores produced in the same culture, and 37 additional mycelia were isolated. Twenty-six of them gave conidia similar to conidia of *Neurospora tetrasperma*. It is a curious fact that 18 of these mycelia were also unisexual and of the same sex as the 11 mycelia previously referred to. The other 8 mycelia were bisexual. Eleven mycelia from spores of this sowing originally produced an abundance of the salmon-pink type of conidia resembling those of *N. sitophila*. Of this number, 10 were unisexual and 1 was bisexual. Of the 10 unisexual mycelia which were judged to produce conidia which were more like *N. sitophila*, 8 mycelia were the same sexually as haplonts B. In every case the classification of a mycelium as to the type of conidia it produced in culture was done long before its sexual nature was known. The data gathered concerning the mycelia from this back-cross combination are given in Table 1. The first 11 mycelia, Nos. 200-210, the ascospores which were not measured, are not included, as they were all of the same sex, haplonts A, and produced scarcely any conidia. As noted in connection with the table, certain mycelia which originally produced conidia in some abundance, when transferred to fresh tubes, developed conidia more like *tetrasperma*. Whether or not further culture work proves the inconsistency of any conclusion which one might be led to draw from an interpretation of the table, it is interesting that the scantiness or abundance with which these back-cross mycelia develop conidia should be even remotely correlated with any sex character.

TABLE 1.—First back-cross ( $f_1 \times p_2$ ) ascospores and their mycelia

No.	Size of Spore ( $\mu$ )	Type of conidia	Sexuality	No.	Size of spore ( $\mu$ )	Type of conidia	Sexuality
300	26.2 by 10.8...	Scanty.....	Unisexual, A.	319	23.4 by 10.8...	Scanty+.....	Unisexual, A.
301	23.4 by 10.8...	do.....	Do	320	21.6 by 10.8...	Abundant— <sup>a</sup> .....	Unisexual, B.
302	19.8 by 10.8...	Abundant.....	Unisexual, B.	321	28.8 by 14.4...	Scanty+.....	Bisexual.
303	28.8 by 13.....	do.....	Bisexual.	322	21.6 by 10.8...	do.....	Unisexual, A.
304	23.4 by 9.....	do.....	Unisexual, B.	323	21.6 by 10.....	do.....	Do.
305	19.8 by 10.8...	Abundant <sup>a</sup> .....	Unisexual, A.	324	21.6 by 10.8...	Scanty.....	Do.
306	21 by 10.8...	Scanty.....	Do.	325	18 by 10.8...	do.....	Do.
307	30 by 15.5...	do.....	Bisexual.	326	23 by 14.4...	Abundant.....	Unisexual, B.
308	19.8 by 12.3...	Abundant— <sup>a</sup> .....	Unisexual, B.	327	21.6 by 10.8...	Abundant— <sup>a</sup> .....	Do.
309	19.8 by 11.5...	Scanty+.....	Unisexual, A.	328	23.4 by 11.....	Abundant.....	Do.
310	21.6 by 10.8...	Abundant.....	Unisexual, B.	329	28.8 by 18.....	Scanty+.....	Bisexual.
311	28.8 by 15.....	Scanty+.....	Bisexual.	330	28.8 by 14.4...	do.....	Unisexual, A.
312	21.6 by 10.8...	Scanty.....	Unisexual, A.	331	28.8 by 14.4...	do.....	Bisexual.
313	27.2 by 14.4...	Scanty+.....	Bisexual.	332	28.8 by 14.4...	Scanty.....	Do.
314	21 by 10.....	do.....	Unisexual, A.	333	21.6 by 10.8...	Scanty+.....	Unisexual, A.
315	23.4 by 10.8...	Abundant— <sup>a</sup> .....	Do.	334	20.5 by 10.8...	Scanty.....	Do.
316	24.5 by 10.8...	Scanty.....	Do.	335	21.6 by 9.....	Scanty+.....	Do.
317	21.6 by 10...	Scanty+.....	Do.	336	34.2 by 14.4...	do.....	Bisexual.
318	20 by 10.8...	do.....	Do.				

\* The later subcultures showed only a scanty development of conidia.

The table shows that, with one exception, all of the mycelia which are unisexual were derived from spores under 27  $\mu$  long. Spores which approach in size ascospores of the *tetrasperma* parent are commonly bisexual. Further work will be necessary to learn just why most of the small spores whose mycelia develop few conidia are all of the same sex.

Each of the 28 mycelia (Nos. 200 to 210 in addition to the unisexual haplont A mycelia, listed in Table 1) derived from ascospores of the first back-cross mating (pl. 3, A) was mated with  $S_1$  of the ancestral parent *tetrasperma*. Most of the matings resulted in the development of asci which as a rule have more than 4 spores. The asci from the mating  $207 \times p_2$  (pl. 3, C) contain mostly 5 or 6 spores. The asci in some perithecia from the mating  $208 \times p_2$  are quite regularly 4 spored (pl. 3, E); in others 5-spored asci with one or two aborted spores are not uncommon (pl. 3, D). Mating  $209 \times p_2$  seems to show that mycelium No. 209 is so nearly like that of  $p_2$  in its inheritance that the asci produced by the mating (pl. 4, B) are just as regularly 4 spored as are those of *Neurospora tetrasperma*. In fact, one can find, if persistent, perithecia of *N. tetrasperma* in which two or three 5-spored asci are maturing. (Pl. 4, A.) Of the two perithecia shown in this illustration (pl. 4, A and B), the asci resulting from the second back cross are more regularly 4 spored than are the asci of the ancestor,  $p_2$ .

The importance of selecting small spores when breeding for purity is well illustrated by the results obtained when one tries to recover the *tetrasperma* type from the second-generation back-cross spores. Asci developed in the back-cross culture  $208 \times p_2$  frequently contain, as noted, only four spores. Twenty-six monosporous mycelia, Nos. 400 to 425, were obtained by germinating spores which for the most part came from four-spored asci. When the perithecia developed there was usually the greatest variability in the number of spores in an ascus. In only two cultures did the majority of the asci have four spores. In most cases asci with five to seven spores predominated. Culture 415 was unisexual.

The recovery of the eight-spored *sitophila* type of perithecia by mating in order mycelia of opposite sex, first from the  $f_1$ , then from the  $f_2$  hybrid ascospores, together with the recovery of the four-spored *tetrasperma* type by back crossing, may be looked upon as evidence that these fungi behave in crossing according to Mendelian principles. The remarkable increase in fertility, however, when the recovered *sitophila* type is again crossed with the original *tetrasperma* parent must be taken to indicate that the recovered type is not exactly like the ancestral form of *Neurospora sitophila* unless as a result of the crossing the change is merely one of increased fertility. The same is true with regard to crosses between the original *sitophila* type and the recovered *tetrasperma* type. No doubt, by continued selection along the lines indicated, strains may be recovered which are physiologically as well as morphologically like the original parents.

The importance of the discovery of species of fungi which can be hybridized so readily is increased by the fact that these species at the same time possess such striking diagnostic characters that they are beautifully adapted for the study of the principles of heredity as applied to the fungi. It has taken several decades and great effort on the part of a number of workers to convince the conservatives of the botanic world that certain species of fungi still retain the power to reproduce themselves sexually. No one can observe the most astonishing variability in types of asci developed in the back crosses referred to previously, contrasting this with the great uniformity in ascus

types of the parent species, and still doubt that nuclei from each of the parents had come together and fused in each  $F_1$  ascus. Since there can be no doubt that species of the genus *Neurospora* can be hybridized in the laboratory there is no reason why crossing should not occur in nature. What this may mean as bearing on the great problems of taxonomy and pathology is evident without further discussion at this time.

#### SUMMARY

Fertile hybrid perithecia have been produced in culture by crossing two species of the red bread-mold fungi *Neurospora sitophila* and *N. tetrasperma*. The asci of the heterothallic form, *N. sitophila*, are regularly eight spored; and the asci of the homothallic species, *N. tetrasperma*, are commonly four spored, although occasionally asci which mature five or six spores are formed. In this case the smaller spores will be unisexual.

The hybrid perithecia resemble in many respects the fruit bodies of *Neurospora sitophila*. Eight spores are commonly delimited in the asci of the hybrid ascocarps, although asci in which only six or seven spores were delimited have been found. There is great irregularity in the size, markings, time of ripening, and number of spores matured in the ascus. As a rule not all of the spores in an ascus are matured.

The first-generation hybrid mycelia usually develop an abundance of conidia of the *sitophila* type. When eight spores are delimited in an ascus they are all unisexual. In the number of ascospores delimited, size of the spores, and type of conidia produced, the first-generation ( $f_1$ ) hybrids are predominantly like the *sitophila* parent.

Second-generation hybrid perithecia have been produced by mating certain mycelia derived from the first-generation hybrid ascospores. The asci in this second-generation cross are very commonly eight spored, each spore being unisexual. Rarely one finds asci with seven spores, one of which is larger and presumably bisexual. Second-generation hybrid mycelia also show some differences in the type of conidia produced.

By mating selected  $f_2$  mycelia of opposite sex, third-generation hybrid ascocarps have been developed. These structures would not be distinguished morphologically from ascocarps of the ancestral parent,  $p_1$ , *Neurospora sitophila*. Behavior of mycelia from their ascospores show, however, that the *sitophila* type recovered is not exactly like the ancestral parent.

The *tetrasperma* type of perithecium has been recovered by back crossing certain first-generation ( $f_1$ ) hybrid mycelia with the  $p_2$  parent form, *N. tetrasperma*, and then again back crossing mycelia derived from ascospores produced in the first back cross with the  $p_2$  parent type. Perithecia resulting from certain of these matings regularly show asci with four spores, and these structures resemble morphologically ascocarps of *Neurospora tetrasperma*.

Although in their morphology the recovered types seem to resemble their ancestral parents, the purity of the segregations developed so far is questioned.

## LITERATURE CITED

- (1) ALLEN, C. E.  
1924. GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS. I. INTRACLONAL VARIATION, AND THE INHERITANCE OF THE TUFTED CHARACTER. *Genetics* 9: 530-587, illus.
- (2) BAUCH, R.  
1927. RASSENUNTERSCHIEDE UND SEKUNDÄRE GESCHLECHTSMERKMALE BEIM ANTHIERENBRAND. *Biol. Zentbl.* 47: 370-383, illus.
- (3) BURGEFF, H.  
1924. UNTERSUCHUNGEN ÜBER SEXUALITÄT UND PARASITISMUS BEI MUCORINEEN I. 135 p., illus. Jena. (Bot. Abhandl. Goebel, Heft 4).
- (4) DICKINSON, S.  
1927. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI, HYPHAL-FUSION. *Roy. Soc. [London] Proc. (B)* 101: 126-136, illus.
- (5) DODGE, B. O.  
1927. NUCLEAR PHENOMENA ASSOCIATED WITH HETEROETHALLISM AND HOMOTHALLISM IN THE ASCOMYCETE NEUROSPORA. *Jour. Agr. Research* 35: 289-305, illus.
- (6) ———  
1928. SPORE FORMATION IN ASCI WITH FEWER THAN EIGHT SPORES. *Mycologia* 20: 18-21.
- (7) KNIEP, H.  
1926. ÜBER ARTKREUZUNGEN BEI BRANDPILZEN. *Ztschr. Pilzk.* (n. s. 5) 10: 217-252, illus.
- (8) SAITO, K., and NAGANISKI, H.  
1915. BEMERKUNGEN ZUR KREUZUNG ZWISCHEN VERSCHIEDENEN MUCOR-ARTEN. *Bot. Mag. [Tokyo]* 29: 149-154, illus.
- (9) SHEAR, C. L., and DODGE, B. O.  
1927. LIFE HISTORIES AND HETEROETHALLISM OF THE RED BREAD-MOLD FUNGI OF THE MONILIA SITOPHILA GROUP. *Jour. Agr. Research* 34: 1019-1042, illus.
- (10) VANDENDRIES, R.  
1923. RECHERCHES SUR LE DÉTERMINISME SEXUEL DES BASIDIOMYCÈTES. *Acad. Roy. Belg. Mém., ser. 2, v. 5, fasc. 1*, 98 p., illus.
- (11) WILCOX, M. S.  
1928. THE SEXUALITY AND ARRANGEMENT OF THE SPORES IN THE ASCUS OF NEUROSPORA SITOPHILA. *Mycologia* 20: 3-17, illus.
- (12) ZÄTTLER, F.  
1924. VERERBUNGSSTUDIEN AN HUTPILZEN (BASIDIOMYZETEN). *Ztschr. Bot.* 16: 433-499, illus.



## FURTHER STUDIES ON THE PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD<sup>1</sup>

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### INTRODUCTION

The fruitfulness of the biometric method of attack on the problem of field heterogeneity—one of the greatest sources of difficulty in the interpretation of the results of all experiments involving plot tests—has been shown by a series of papers cited in publications readily accessible to agriculturists (2, 3, 4, 5, 6).<sup>2</sup>

In an earlier investigation (7) the writers considered the permanence of the differences in the plots of one experimental tract, comprising 46 plots, at the Huntley (Mont.) Field Station.

By applying the method of interannual correlation (3) the writers demonstrated that during the period from 1911 to 1919, inclusive, plots which showed a higher yield in one year were generally characterized by a measurably higher yield in subsequent years, whereas those which fell below the average yield in a given year generally proved measurably inferior in other years.

The crops grown during the nine years covered by the first investigation comprised sugar beets, alfalfa, ear corn, oats, silage corn, and barley. Altogether, 19 crop records (including the various cuttings of alfalfa and the separate and combined yields of grain and straw, in the cereals) were available. Since the report of these investigations appeared, 6 crops, giving 10 measures of yield, have been grown on this land. These were silage corn, barley, alfalfa (three years), and silage corn.

The purposes of the present investigation are: (1) To consider the relationship between the yield of these various crops and the other crops grown on these plots during the whole period that the uniform-cropping experiments were under way—that is, from 1911 to 1925, inclusive; (2) to suggest physical explanations for certain of the biological results; and (3) to indicate the bearing of these results on the problem of the technic of agricultural experimentation.

### MATERIALS

While the experimental tract was briefly described in an earlier paper (7), certain of the features must be noted in somewhat greater detail to make possible a full understanding of the results here presented.

<sup>1</sup> Received for publication Sept. 17, 1927; issued February, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 40.



Some slope of the land is necessary for the distribution of water, but it is found to be difficult to establish and maintain a uniform surface slope over the entire field. An effort is made to smooth the surface each time the land is prepared for a new crop, but slight inequalities of slope may persist. Furthermore, some soil movement may take place through wind action between the time the field is leveled for planting and the time the crop is grown to a size that affords protection for the soil. Even when the land is well stabilized by a crop such as alfalfa, wind may carry soil from adjacent fields or roads, thus making the surface to some extent uneven. Any such irregularities may have a direct influence on the distribution of irrigation water and consequently on the depth of penetration even where the physical conditions of the soil of the root zone are substantially uniform. These irregularities in the soil surface must have some influence upon the character of the crop growth. Growth conditions may be affected adversely either where the surface is slightly high, so that too little water enters the root zone, or where the surface is slightly low, so that water collects and keeps the surface soil saturated too long. The indications are that, with the quantities of irrigation water that have been used on this field, more injury has resulted from local deficiencies of water than from local surpluses. These differences of surface level do not occur in the same spots from year to year, because they are the result of accidental conditions. Consequently, differences of yield from year to year may be due in part to these slight surface irregularities.

The question of the relation of the field to water supply and drainage—factors which are believed to be of importance in determining some of the results reported here—will now be considered.

The southeast corner of Series II, the east series, is about 80 feet from the main canal, and the southwest corner of Series III is about 50 feet from Custer Coulee. The main project canal carries normally during the irrigation season about 400 second-feet of water. The water surface in the canal is about 4 feet above the high corner of the field. It is evident from surface conditions, as well as from borings made between the canal and the field, that there is extensive seepage from the canal into the subsoil of the field. The volume of this seepage has been larger in recent years than it was in the earlier years of the cropping experiments, probably because the canal bank has been worn away by internal erosion, exposing a stratum of sandy subsoil that underlies the canal and part of the field.

As early as 1921 it was observed that in some parts of the field the subsoil was saturated late in the season not far below the surface. In the spring of 1922 an observation well was established near the center of the south line of the field, at the end of the alleyway that separates the two series. In April, when the well was put down, the saturated zone was 7 feet below the surface. During the irrigation season the underground water rose until in October it stood at 2.1 feet below the ground surface. Each year since 1922 a similar condition has been observed. The underground water rises during the irrigation season and falls again during the following winter. Other observation wells were sunk in 1923 along the east and north sides of the field. From these it has been learned that the zone of

subsoil saturation extends under the whole field. The slope of this saturated zone is rather greater than that of the surface soil, so that while it rises nearly to the soil surface along the east side of the field (Series II) it remains 4 to 5 feet below the surface along the west side. It seems obvious that this zone of saturated subsoil as it approaches the surface must restrict the roots of the plants and to some extent influence crop growth. At some stages this influence may be beneficial, since the plants may be able to utilize this subsoil water. At higher stages or with such a deep-rooted crop as alfalfa the influence may be injurious.

It is believed that in the earlier years of these uniform-cropping experiments the subsoil water did not rise so close to the surface as it has recently, although no detailed observations were made prior to 1922. It is also believed that the effects of the subsoil saturation have been more pronounced on the plots of Series II than on those of Series III. It seems probable that the negative correlations reported in this paper between the alfalfa yields of 1912-1914 and those of 1922-1924 may be associated with the unequal effect on the two series of plots of the rising zone of subsoil saturation.

The following notes with respect to the field conditions and crops grown each year may contribute to a better understanding of the yields reported and analyzed in this paper.

The detailed history of the field prior to 1910 is not definitely known. Probably it was first broken from the original prairie sod in the spring of 1908. In 1909 it was planted to sugar beets, but the crop was destroyed by hail in the late summer. In 1910 the field came under experimental control. At that time the major portion was sown to oats, which produced a yield of 66 bushels per acre. That year a small tract in the southeast corner of the field was used as an implement park and stack yard and was not put into crops. This area occupied about two-thirds of the length of the first five plots of Series II. Possibly this difference in treatment in 1910 may have influenced the crop yields of 1911, but it seems probable that such influence was not great.

Year by year the crops were as follows:

1911. Sugar beets. (See above.)

1912. Montana common alfalfa was seeded on May 18 without a nurse crop. The first crop was cut July 18, but the plots were not harvested separately. The total yield from the two series was 6,840 pounds. The second cutting (reported in Table 2 as "1912, alfalfa total") was made September 10.

1913. Alfalfa I was cut June 20; Alfalfa II was cut August 2; the third cutting was made September 21. While this crop was curing in the field a severe wind-storm occurred, and the hay was so badly mixed that the plot yields could not be determined. It was not possible even to obtain the total yield, as part of the hay was blown completely off the field.

1914. Alfalfa I was cut June 15; Alfalfa II was cut July 7; Alfalfa III was cut September 20. The land was subsequently plowed.

1915. Ear corn, variety Northwestern Dent, was planted May 20. Harvest was begun September 20, but was delayed for several weeks by stormy weather. The stover weights were not taken, because of storm injury during harvest.

1916. Ear corn, variety Northwestern Dent, was planted May 26. The soil was very dry at planting time and the crop was slow in starting. Harvesting was delayed by wet weather until December 1, and again the injury to the stover was so great that it seemed inadvisable to weigh it by plots.

1917. Oats, variety Banner, were sown May 28 and harvested August 17. No exceptional conditions were noted. The crop yield was very good.

1918. Silage corn, variety Northwestern Dent, was planted May 20 and cut September 18. No exceptional conditions were noted.

1919. Barley, variety Trebi, was sown May 10 and harvested September 12. Because of drought, an irrigation was necessary to germinate the seed. Only a fair stand was obtained.

1920. Silage corn, variety Northwestern Dent. On July 4 water from Custer Coulee caused an overflow of the main canal and flooded the experimental field. This set back the growth of the corn crop and probably reduced the yield, but so far as could be observed the injury was fairly uniform on the two series. The crop was harvested September 16.

1921. Barley, variety Trebi. A good yield was obtained and approximately a normal grain-straw ratio for this variety.

1922. Alfalfa, variety Grimm, was seeded with the barley in May, 1921, but owing to severe grasshopper injury it was necessary to reseed in August, 1921. In 1922 the first and second cuttings of alfalfa were not harvested by separate plots because of the prevalence and irregular distribution of weeds and grasshopper injury. The alfalfa reported for 1922 in Table 2 is the third crop, cut September 27. The yields for the first cutting, June 23, were: Series II, 8,090 pounds, or 1.03 tons per acre; Series III, 7,000 pounds, or 0.90 ton per acre. The yields for the second cutting, August 4, were: Series II, 8,050 pounds, or 1.03 tons per acre; Series III, 7,950 pounds, or 1.02 tons per acre.

1923. Alfalfa. The first cutting was made June 15. The second cutting, made August 3, was so badly lodged by wind and rain that it was not practicable to weigh the plot yields separately. The yield of Series II was 15,730 pounds, or 2.01 tons per acre; the yield of Series III was 16,911 pounds, or 2.16 tons per acre. The third cutting was not made until November 20, about six weeks after it was ready to cut. This delay was due to continuously unfavorable weather, and although there was appreciable loss of weight because of the delay it was not apparent that any lack of uniformity in yield resulted.

1924. Alfalfa. The first crop was cut June 25. It was so badly lodged by wind and rain that the plots could not be weighed separately. Series II yielded 18,660 pounds, or 2.39 tons per acre, and Series III yielded 19,500 pounds, or 2.50 tons per acre. The second cutting was made August 5. The third cutting was made September 26. The hay from plots B-II, 1 to 14, inclusive, was weighed on October 2. The hay from the remaining plots of B-II and all the other plots was weighed October 16. The delay was due to a heavy rain, which may have caused some loss of leaves occasioned by the necessary turning and drying.

1925. Silage corn, variety Northwestern Dent, harvested September 12. Apparently a normal crop and good yield.

The yields of the various crops grown during the period 1911-1919 are set forth in Table 3 of a previous paper (7). Those for the period 1920-1925 are shown in Table 1 of the present paper. This table gives the yields in terms of pounds per plot. For the convenience of agriculturists the average yields in pounds per plot and the equivalent yields per acre in conventional units are shown in Table 2.

TABLE 1.—Crop yields in the uniform-cropping experiments at Huntley, Mont., 1920-1925

		Yield per plot (pounds)								
Series and plot No.	Silage corn, 1920	Barley, 1921			Alfalfa					Silage corn, 1925
		Grain	Straw	Total	III, 1922	I, 1923	III, 1923	II, 1924	III, 1924	
Series II:										
1.....	2,230	506	344	850	420	530	270	450	250	4,940
2.....	2,405	571	389	960	520	450	220	460	290	3,820
3.....	2,800	554	376	930	390	640	280	550	280	3,570
4.....	2,720	559	361	920	340	510	260	500	330	3,600
5.....	2,470	569	381	950	390	620	290	650	290	4,150
6.....	2,290	512	388	900	390	540	250	540	340	4,490
7.....	2,320	552	348	900	400	600	310	680	280	4,280
8.....	2,335	514	326	840	390	510	250	530	310	4,120
9.....	2,380	499	341	840	410	540	290	670	330	4,400
10.....	2,655	548	372	920	490	545	280	590	370	4,110
11.....	2,410	559	341	900	480	730	360	700	310	4,760
12.....	2,560	598	312	910	500	610	280	610	400	4,510
13.....	2,490	547	303	850	450	670	330	740	400	4,600
14.....	2,560	550	280	830	440	570	290	630	450	4,140
15.....	2,300	509	261	770	400	660	410	800	380	4,150
16.....	2,590	566	334	900	430	560	300	640	430	4,720
17.....	2,490	634	416	1,050	420	630	310	700	350	4,550
18.....	2,550	616	374	990	430	550	250	550	370	4,340
19.....	2,610	568	342	910	340	640	310	600	280	4,390
20.....	2,430	571	379	950	360	630	260	560	230	4,420
21.....	2,180	546	344	890	320	640	290	650	290	4,520
22.....	2,360	521	239	760	230	550	210	530	310	4,320
23.....	1,790	452	288	740	200	540	240	530	260	4,180
Series III:										
1.....	2,240	466	304	770	260	645	350	720	360	4,690
2.....	2,110	485	325	810	330	540	360	660	410	3,590
3.....	2,535	519	351	870	340	750	370	720	380	3,070
4.....	2,480	493	307	800	350	490	320	590	410	3,140
5.....	2,300	498	342	840	395	670	340	790	400	3,940
6.....	2,215	436	334	770	350	590	340	640	350	3,960
7.....	2,150	502	338	840	420	720	330	800	400	4,170
8.....	2,220	497	303	800	390	500	360	660	420	3,880
9.....	2,400	476	304	780	395	760	320	820	390	3,970
10.....	2,460	489	311	800	305	590	290	620	420	3,270
11.....	2,245	465	305	770	290	620	360	780	380	4,105
12.....	2,330	492	298	790	410	550	340	550	390	4,495
13.....	2,400	506	304	810	380	600	280	750	330	3,825
14.....	2,430	526	314	840	370	550	270	590	360	3,390
15.....	2,300	523	297	820	340	630	290	690	340	4,380
16.....	2,530	501	289	790	305	500	280	530	380	4,500
17.....	2,540	515	305	820	380	640	300	670	360	4,520
18.....	2,480	537	273	810	365	600	240	570	390	4,200
19.....	2,610	505	285	790	375	740	290	720	380	4,620
20.....	2,610	531	309	840	370	650	310	600	420	4,510
21.....	2,480	542	288	830	440	640	340	790	400	4,770
22.....	1,940	523	317	840	345	680	270	620	420	4,850
23.....	2,200	530	300	830	410	670	320	810	420	5,230

TABLE 2.—Average yields of crops grown in the uniform-cropping experiments at Huntley, Mont., 1911–1925

[Yields per acre (last column) are stated in tons except for ear corn, barley grain, and oats grain, which are in bushels]

Year	Crop	Per plot (pounds)	Per acre
1911	Sugar beets.....	4, 179 00	12. 29
1912	Alfalfa (total).....	356. 54	1. 04
	Alfalfa I.....	541. 41	1. 59
1913	Alfalfa II.....	483. 26	1. 42
	Alfalfa I and II.....	1, 024. 67	3. 01
	Alfalfa I.....	489. 13	1. 44
	Alfalfa II.....	499. 34	1. 47
1914	Alfalfa I and II.....	988. 47	2. 91
	Alfalfa III.....	471. 95	1. 38
	Alfalfa I to III.....	1, 460. 43	4. 29
1915	Ear corn.....	522. 58	42. 70
1916	do.....	396. 15	32. 40
	Grain.....	555. 80	102. 10
1917	Oats Straw.....	521. 54	1. 53
	Total.....	1, 077. 34	3. 16
1918	Silage corn.....	3, 175. 43	9. 34
	Grain.....	358. 19	43. 80
1919	Barley Straw.....	230. 50	. 67
	Total.....	588. 69	1. 73
1920	Silage corn.....	2, 394. 13	7. 04
	Grain.....	525. 60	64. 20
1921	Barley Straw.....	324. 82	. 95
	Total.....	850. 43	2. 50
1922	Alfalfa III.....	379. 67	1. 12
	Alfalfa I.....	603. 91	1. 77
1923	Alfalfa III.....	300. 22	. 88
	Alfalfa I and III.....	904. 13	2. 66
	Alfalfa II.....	642. 39	1. 89
1924	Alfalfa III.....	357. 39	1. 05
	Alfalfa II and III.....	999. 78	2. 94
1925	Silage corn.....	4, 267. 93	12. 55

## PRESENTATION OF RESULTS

The correlations between the crops grown from 1911 to 1919, inclusive, have been set forth in Tables 4 to 6 of an earlier publication (7).

With the new materials at hand it is worth while to review all of the correlations available for the entire period of 15 years over which these uniform-cropping experiments have been continued.

The correlations between each crop yield of the period 1920 to 1925 and the yields of each of the crops grown from 1911 to 1925, both inclusive, are shown in Table 3.

The publication of one of the present tables in condensed form and the appearance of these tables in two different places renders the examination of the results extremely difficult. A graphic method of representation, therefore, has been adopted.

TABLE 3.—Correlation between the yields of various crops in the uniform-cropping experiments at Huntley, Mont.

Year	Crop	Item	Sugar beets, 1911	Alfalfa							
				Total, 1912	I, 1913	II, 1913	I and II, 1913	I, 1914	II, 1914	I and II, 1914	
1920	Silage corn.	Correlation.....	{ -0.237	+0.074	+0.117	+0.189	+0.179	+0.164	+0.164	+0.177	
		r/Er.....	{ ± 0.093	± 0.098	± 0.098	± 0.095	± 0.096	± 0.096	± 0.096	± 0.096	
	Grain.....	Correlation.....	{ +0.081	± 0.001	± 0.473	± 0.219	± 0.408	± 0.529	± 0.286	± 0.432	
		r/Er.....	{ ± 0.098	± 0.099	± 0.077	± 0.094	± 0.082	± 0.071	± 0.091	± 0.080	
1921	Barley	Correlation.....	{ -0.350	± 0.219	± 0.415	± 0.189	± 0.356	± 0.492	± 0.408	± 0.484	
	Straw.....	r/Er.....	{ ± 0.087	± 0.094	± 0.082	± 0.095	± 0.086	± 0.075	± 0.082	± 0.076	
	Total.....	Correlation.....	{ 4.02	2.31	5.04	1.97	4.10	6.54	4.93	6.36	
		r/Er.....	{ ± 0.144	± 0.121	± 0.515	± 0.237	± 0.443	± 0.591	± 0.398	± 0.528	
1922	Alfalfa III.....	r/Er.....	{ ± 0.097	± 0.097	± 0.073	± 0.093	± 0.079	± 0.064	± 0.083	± 0.071	
	Alfalfa I.....	Correlation.....	{ 1.48	1.24	7.05	2.52	5.55	9.15	4.76	7.38	
		r/Er.....	{ -0.197	± 0.195	± 0.056	± 0.203	± 0.151	± 0.292	± 0.115	± 0.214	
	Alfalfa II.....	Correlation.....	{ ± 0.095	± 0.095	± 0.099	± 0.095	± 0.097	± 0.090	± 0.098	± 0.094	
		r/Er.....	{ 2.06	2.04	5.97	2.13	1.55	3.21	1.18	2.26	
	Alfalfa I and II.....	Correlation.....	{ ± 0.082	± 0.011	± 0.060	± 0.207	± 0.159	± 0.224	± 0.204	± 0.265	
		r/Er.....	{ ± 0.098	± 0.099	± 0.099	± 0.095	± 0.096	± 0.094	± 0.092	± 0.092	
	Alfalfa III.....	Correlation.....	{ 83	1.11	6.7	2.17	1.64	2.38	2.86	2.87	
		r/Er.....	{ -0.081	± 0.150	± 0.224	± 0.333	± 0.326	± 0.396	± 0.488	± 0.452	
	Alfalfa I and II.....	Correlation.....	{ ± 0.098	± 0.097	± 0.094	± 0.088	± 0.088	± 0.083	± 0.080	± 0.079	
		r/Er.....	{ 82	1.54	2.37	3.77	3.67	4.73	5.45	5.72	
	Alfalfa I and II.....	Correlation.....	{ +0.025	± 0.055	± 0.143	± 0.292	± 0.254	± 0.332	± 0.379	± 0.386	
		r/Er.....	{ ± 0.099	± 0.099	± 0.097	± 0.090	± 0.092	± 0.088	± 0.085	± 0.084	
	Alfalfa II.....	Correlation.....	{ 26	55	1.47	3.22	2.73	3.75	4.45	4.56	
		r/Er.....	{ +0	± 0.099	± 0.099	± 0.092	± 0.085	± 0.083	± 0.085	± 0.082	
1924	Alfalfa III.....	r/Er.....	{ 0	1.00	2.95	4.19	4.33	4.69	4.30	4.99	
	Alfalfa I.....	Correlation.....	{ -0.026	± 0.047	± 0.556	± 0.279	± 0.492	± 0.540	± 0.544	± 0.586	
		r/Er.....	{ ± 0.099	± 0.099	± 0.068	± 0.091	± 0.075	± 0.070	± 0.069	± 0.065	
	Alfalfa II and III.....	Correlation.....	{ 26	48	8.09	3.04	6.53	7.67	7.77	8.98	
		r/Er.....	{ -0.100	± 0.054	± 0.435	± 0.388	± 0.483	± 0.520	± 0.503	± 0.553	
	Alfalfa I and II.....	Correlation.....	{ ± 0.099	± 0.099	± 0.080	± 0.084	± 0.076	± 0.072	± 0.074	± 0.069	
		r/Er.....	{ 10	54	5.41	4.59	6.34	7.18	6.79	8.01	
1925	Silage corn.....	Correlation.....	{ ± 0.512	± 0.572	± 0.131	± 0.096	± 0.134	± 0.074	± 0.022	± 0.051	
		r/Er.....	{ ± 0.073	± 0.066	± 0.097	± 0.098	± 0.097	± 0.098	± 0.099	± 0.099	
			{ 6.99	8.57	1.34	0.98	1.37	0.75	0.20	0.51	
Year	Crop	Item	Alfalfa—Continued		Ear corn		Oats			Silage corn, 1918	
			III, 1914	I to III, 1914	1915	1916	Grain, 1917	Straw, 1917	Total, 1917		
1920	Silage corn.	Correlation.....	{ +0.111	+0.171	-0.478	+0.105	+0.251	+0.983	+0.185	-0.152	
		r/Er.....	{ ± 0.098	± 0.096	± 0.076	± 0.098	± 0.093	± 0.098	± 0.096	± 0.097	
	Grain.....	Correlation.....	{ 1.13	1.77	6.24	1.07	2.69	84	1.93	1.56	
		r/Er.....	{ ± 0.248	± 0.411	± 0.131	± 0.401	± 0.268	± 0.143	± 0.233	± 0.011	
	Barley	Correlation.....	{ ± 0.093	± 0.082	± 0.097	± 0.083	± 0.092	± 0.097	± 0.093	± 0.099	
		r/Er.....	{ 2.65	4.97	1.34	4.81	2.90	1.47	2.54	1.11	
1921	Straw.....	Correlation.....	{ +0.343	± 0.048	± 0.085	± 0.400	± 0.331	± 0.278	± 0.371	± 0.036	
		r/Er.....	{ ± 0.087	± 0.099	± 0.099	± 0.083	± 0.088	± 0.091	± 0.085	± 0.099	
	Total.....	Correlation.....	{ 3.91	4.48	3.35	4.79	3.74	3.03	4.31	3.36	
		r/Er.....	{ +0.339	± 0.573	± 0.098	± 0.403	± 0.345	± 0.240	± 0.348	± 0.027	
1922	Alfalfa III.....	r/Er.....	{ ± 0.087	± 0.073	± 0.098	± 0.078	± 0.087	± 0.093	± 0.087	± 0.099	
	Alfalfa I.....	Correlation.....	{ 3.85	7.00	1.00	5.93	3.93	2.56	3.90	2.27	
		r/Er.....	{ +0.057	± 0.184	± 0.058	± 0.192	± 0.148	± 0.397	± 0.369	± 0.126	
	Alfalfa II.....	Correlation.....	{ ± 0.090	± 0.096	± 0.099	± 0.095	± 0.097	± 0.083	± 0.085	± 0.097	
		r/Er.....	{ 57	1.91	5.9	2.01	1.53	4.74	4.04	1.09	
	Alfalfa I and II.....	Correlation.....	{ -0.125	± 0.244	± 0.127	± 0.260	± 0.187	± 0.095	± 0.020	± 0.101	
		r/Er.....	{ ± 0.097	± 0.093	± 0.097	± 0.092	± 0.095	± 0.098	± 0.099	± 0.098	
1923	Alfalfa III.....	Correlation.....	{ 1.28	2.61	1.30	2.80	1.95	0.77	2.0	1.03	
		r/Er.....	{ -0.142	± 0.304	± 0.101	± 0.439	± 0.405	± 0.141	± 0.092	± 0.127	
	Alfalfa I.....	Correlation.....	{ ± 0.097	± 0.083	± 0.098	± 0.080	± 0.083	± 0.097	± 0.098	± 0.097	
		r/Er.....	{ 1.45	4.70	1.02	5.47	4.87	1.45	94	1.29	
	Alfalfa I and II.....	Correlation.....	{ -0.152	± 0.346	± 0.136	± 0.376	± 0.308	± 0.130	± 0.054	± 0.021	
		r/Er.....	{ ± 0.097	± 0.097	± 0.097	± 0.085	± 0.089	± 0.097	± 0.099	± 0.099	
	Alfalfa II.....	Correlation.....	{ 1.56	3.95	1.39	4.40	3.43	1.33	0.59	0.21	
		r/Er.....	{ -0.214	± 0.385	± 0.021	± 0.459	± 0.381	± 0.025	± 0.206	± 0.130	
	Alfalfa I and II.....	Correlation.....	{ ± 0.094	± 0.084	± 0.099	± 0.078	± 0.085	± 0.099	± 0.095	± 0.097	
		r/Er.....	{ 2.26	4.54	2.42	5.85	4.43	2.6	2.10	1.33	
1924	Alfalfa III.....	Correlation.....	{ -0.363	± 0.565	+0.422	± 0.661	± 0.430	± 0.040	± 0.181	± 0.154	
		r/Er.....	{ ± 0.086	± 0.067	± 0.099	± 0.068	± 0.081	± 0.099	± 0.090	± 0.097	
	Alfalfa I.....	Correlation.....	{ 4.21	8.35	4.42	8.25	5.30	4.0	1.88	1.08	
		r/Er.....	{ -0.312	± 0.524	± 0.001	± 0.678	± 0.465	± 0.002	± 0.230	± 0.162	
	Alfalfa II and III.....	Correlation.....	{ ± 0.089	± 0.072	± 0.099	± 0.066	± 0.077	± 0.099	± 0.094	± 0.096	
		r/Er.....	{ 3.48	7.20	0.1	8.73	5.96	0.2	2.44	1.67	
1925	Silage corn.....	Correlation.....	{ -0.027	± 0.031	± 0.107	± 0.192	± 0.176	± 0.030	± 0.044	± 0.093	
		r/Er.....	{ ± 0.099	± 0.098	± 0.098	± 0.095	± 0.096	± 0.099	± 0.099	± 0.098	
			{ 28	31	1.09	2.01	1.83	3.0	65	0.98	

Year	Crop	Item	Alfalfa—Continued		Ear corn		Oats			Silage corn, 1918
			III, 1914	I to III, 1914	1915	1916	Grain, 1917	Straw, 1917	Total, 1917	
1920	Silage corn.....	Correlation.....	{ +0.111	+0.171	-0.478	+0.105	+0.251	+0.983	+0.185	-0.152
		r/Er.....	{ ± 0.098 ± 0.096	± 0.076	± 0.098	± 0.093	± 0.098	± 0.096	± 0.097	± 0.097
	Grain.....	Correlation.....	{ 1.13	1.77	6.24	1.07	2.69	8.84	1.93	1.56
		r/Er.....	{ ± 0.248 ± 0.411	± 0.131	± 0.401	± 0.268	± 0.143	± 0.238	± 0.011	± 0.011
1921	Barley.....	Correlation.....	{ ± 0.093 ± 0.082	± 0.097	± 0.083	± 0.092	± 0.097	± 0.093	± 0.093	± 0.093
		r/Er.....	{ 2.65	4.97	1.34	4.81	2.90	1.47	2.54	1.11
	Straw.....	Correlation.....	{ ± 0.343 ± 0.048	± 0.035	± 0.400	± 0.331	± 0.278	± 0.370	± 0.036	± 0.036
		r/Er.....	{ ± 0.087 ± 0.099	± 0.099	± 0.083	± 0.088	± 0.091	± 0.085	± 0.099	± 0.099
1922	Total.....	Correlation.....	{ 3.91	4.48	3.35	4.79	3.74	3.03	4.31	3.36
		r/Er.....	{ ± 0.339 ± 0.573	± 0.098	± 0.463	± 0.345	± 0.240	± 0.348	± 0.027	± 0.027
	Alfalfa III.....	Correlation.....	{ ± 0.087 ± 0.073	± 0.098	± 0.078	± 0.087	± 0.098	± 0.087	± 0.099	± 0.099
		r/Er.....	{ 3.85	7.00	1.00	5.93	3.93	2.56	3.99	0.27
1923	Alfalfa I.....	Correlation.....	{ ± 0.057 ± 0.184	± 0.058	± 0.192	± 0.148	± 0.397	± 0.369	± 0.126	± 0.126
		r/Er.....	{ ± 0.090 ± 0.096	± 0.099	± 0.095	± 0.097	± 0.083	± 0.085	± 0.097	± 0.097
	Alfalfa II.....	Correlation.....	{ 57	1.91	5.59	2.01	1.53	4.74	4.30	1.29
		r/Er.....	{ ± 0.125 ± 0.244	± 0.127	± 0.260	± 0.187	± 0.095	± 0.020	± 0.101	± 0.101
1924	Alfalfa III.....	Correlation.....	{ ± 0.097 ± 0.093	± 0.097	± 0.092	± 0.095	± 0.098	± 0.098	± 0.098	± 0.098
		r/Er.....	{ 1.28	2.61	1.30	2.80	1.95	0.97	2.01	1.03
	Alfalfa I and II.....	Correlation.....	{ -0.142 ± 0.304	± 0.101	± 0.439	± 0.405	± 0.141	± 0.092	± 0.127	± 0.127
		r/Er.....	{ ± 0.097 ± 0.083	± 0.098	± 0.080	± 0.083	± 0.097	± 0.098	± 0.097	± 0.097
1925	Silage corn.....	Correlation.....	{ 1.45	4.70	1.02	5.47	4.87	1.45	0.94	1.29
		r/Er.....	{ ± 0.152 ± 0.346	± 0.136	± 0.376	± 0.308	± 0.130	± 0.054	± 0.021	± 0.021
	Alfalfa II.....	Correlation.....	{ ± 0.097 ± 0.087	± 0.097	± 0.085	± 0.089	± 0.097	± 0.099	± 0.099	± 0.099
		r/Er.....	{ 1.56	3.95	1.39	4.40	3.43	1.33	0.54	0.21
1926	Alfalfa III.....	Correlation.....	{ -0.214 ± 0.385	± 0.021	± 0.459	± 0.381	± 0.025	± 0.206	± 0.130	± 0.130
		r/Er.....	{ ± 0.094 ± 0.084	± 0.099	± 0.078	± 0.085	± 0.099	± 0.095	± 0.097	± 0.097
	Alfalfa I and II.....	Correlation.....	{ 2.26	4.54	2.22	5.85	4.48	2.26	2.16	1.33
		r/Er.....	{ ± 0.363 ± 0.505	± 0.442	± 0.561	± 0.430	± 0.404	± 0.181	± 0.154	± 0.154
1927	Silage corn.....	Correlation.....	{ ± 0.086 ± 0.067	± 0.099	± 0.068	± 0.081	± 0.099	± 0.096	± 0.097	± 0.097
		r/Er.....	{ 4.21	8.35	4.2	8.25	5.30	4.0	1.88	1.58
	Alfalfa II.....	Correlation.....	{ -0.312 ± 0.524	± 0.001	± 0.578	± 0.465	± 0.002	± 0.230	± 0.162	± 0.162
		r/Er.....	{ ± 0.089 ± 0.072	± 0.099	± 0.066	± 0.077	± 0.099	± 0.094	± 0.096	± 0.096
1928	Silage corn.....	Correlation.....	{ 3.48	7.20	0.01	8.73	5.96	0.02	2.44	1.67
		r/Er.....	{ ± 0.027 ± 0.031	± 0.107	± 0.192	± 0.176	± 0.030	± 0.064	± 0.093	± 0.093
	Alfalfa I.....	Correlation.....	{ ± 0.099 ± 0.099	± 0.098	± 0.095	± 0.096	± 0.099	± 0.099	± 0.099	± 0.099
		r/Er.....	{ 28	31	1.09	2.01	1.83	0.30	0.65	0.95



TABLE 3.—Correlation between the yields of various crops in the uniform-cropping experiments at Huntley, Mont.—Continued

Year	Crop	Item	Barley			Silage corn, 1920	Barley		
			Grain, 1919	Straw, 1919	Total, 1919		Grain, 1921	Straw, 1921	Total, 1921
1920	Silage corn	Correlation	-0.092	-0.274	-0.195		+0.532	+0.206	+0.435
		r/Er	±.098	±.091	±.095		±.071	±.095	±.080
			.93	2.98	2.04		7.46	2.16	5.39
		Correlation	-0.184	-0.282	-0.253	+0.532		+0.495	+0.877
1921	Barley	Grain	±.096	±.091	±.093	±.071		±.075	±.022
		r/Er	1.92	3.08	2.72	7.46		6.60	38.3
		Straw	±.348	±.203	±.308	±.206	+0.495		+0.851
		Total	±.087	±.095	±.090	±.095	±.075		±.027
1922	Alfalfa III	Correlation	3.99	2.13	3.42	2.16	6.60		31.2
		r/Er	±.081	-0.057	+0.017	+0.435	+0.877		±.851
			±.098	±.099	±.099	±.099	±.022		±.027
		r/Er	.82	.58	.17	5.39	38.3	31.2	
1923	Alfalfa I	Correlation	+0.282	+0.184	+0.259	+0.376	+0.534	+0.372	+0.528
		r/Er	±.091	±.096	±.092	±.085	±.071	±.085	±.071
			3.08	1.92	2.80	4.41	7.51	4.35	7.36
		Correlation	-0.021	-0.001	-0.012	+0.018	+0.005	-0.091	-0.047
1924	Alfalfa II	Correlation	±.099	±.099	±.099	±.099	±.099	±.098	±.099
		r/Er	.21	.01	.12	.18	.05	.93	.47
			+0.232	+0.078	+0.175	-0.109	-0.296	-0.196	-0.265
		r/Er	±.094	±.098	±.096	±.098	±.090	±.096	±.092
1925	Alfalfa III	Correlation	2.47	.79	1.82	1.11	3.26	2.05	2.87
		r/Er	±.082	+0.032	+0.064	-0.032	-0.120	-0.133	-0.146
			±.098	±.099	±.099	±.099	±.097	±.097	±.097
		r/Er	.83	.33	.65	.32	1.23	1.37	1.50
1926	Alfalfa I and III	Correlation	+0.130	+0.065	+0.109	-0.144	-0.193	-0.299	-0.249
		r/Er	±.097	±.099	±.098	±.097	±.095	±.093	±.093
			1.33	.65	1.11	1.47	2.02	2.55	2.67
		Correlation	-0.024	-0.058	-0.047	+0.073	-0.155	-0.411	-0.321
1927	Alfalfa II	Correlation	±.099	±.099	±.098	±.098	±.097	±.082	±.080
		r/Er	.24	.58	7.85	7.4	1.60	4.97	8.60
			+0.108	+0.024	-0.146	-0.077	-0.209	-0.351	-0.320
		r/Er	±.098	±.099	±.097	±.098	±.095	±.087	±.089
1928	Alfalfa II and III	Correlation	1.10	.24	1.50	.78	2.20	4.02	3.59
		r/Er	±.098	±.099	±.097	±.098	±.095	±.087	±.089
			-0.283	-0.191	-0.535	-0.167	+0.194	-0.152	+0.033
		r/Er	±.091	±.095	±.070	±.096	±.095	±.097	±.099
1929	Silage corn	Correlation	3.10	1.99	7.54	1.73	2.02	1.57	.33
		r/Er							

Year	Crop	Item	Alfalfa							Silage corn, 1925
			III, 1922	I, 1923	III, 1923	I and III, 1923	II, 1924	III, 1924	II and III, 1924	
1920	Silage corn	Correlation	+0.376	+0.018	-0.109	-0.032	-0.144	+0.073	-0.077	-0.167
		r/Er	±.085	±.099	±.098	±.099	±.097	±.098	±.088	±.096
			4.41	.18	1.11	.32	1.47	.74	.78	1.73
		Correlation	+0.534	+0.005	-0.296	-0.120	-0.193	-0.155	-0.209	+0.194
1921	Barley	Grain	±.071	±.099	±.090	±.097	±.095	±.097	±.095	±.095
		r/Er	7.51	.05	3.26	1.23	2.02	1.60	2.20	2.02
		Straw	±.372	-0.091	-0.157	-0.133	-0.239	-0.411	-0.351	-0.152
		Total	±.085	±.098	±.096	±.097	±.093	±.082	±.087	±.097
1922	Alfalfa III	Correlation	4.35	.93	1.62	1.37	2.55	4.97	4.02	1.57
		r/Er	±.528	-0.047	-0.265	-0.146	-0.249	-0.321	-0.320	+0.033
			±.071	±.099	±.092	±.097	±.093	±.089	±.089	±.099
		r/Er	7.36	.47	2.87	1.50	2.67	3.60	3.59	.33
1923	Alfalfa I	Correlation	±.027	±.027	±.069	±.049	+0.074	+0.147	+0.117	+0.185
		r/Er	.28	.09	±.098	±.099	±.098	±.097	±.098	±.096
			+0.457	+0.926	±.014	±.048	±.057	±.063	±.096	
		Correlation	±.089	±.078	±.082	±.082	±.082	±.082	±.082	±.082
1924	Alfalfa II	Correlation	±.089	±.078	±.082	±.082	±.082	±.082	±.082	±.082
		r/Er	.09	.81	±.082	±.082	±.082	±.082	±.082	±.082
			+0.049	+0.926	±.082	±.082	±.082	±.082	±.082	±.082
		Correlation	±.089	±.078	±.082	±.082	±.082	±.082	±.082	±.082
1925	Alfalfa III	Correlation	±.089	±.078	±.082	±.082	±.082	±.082	±.082	±.082
		r/Er	.50	.66	±.082	±.082	±.082	±.082	±.082	±.082
			+0.074	+0.717	±.082	±.082	±.082	±.082	±.082	±.082
		Correlation	±.098	±.048	±.049	±.031	±.031	±.031	±.031	±.031
1926	Alfalfa I and III	Correlation	±.098	±.048	±.049	±.031	±.031	±.031	±.031	±.031
		r/Er	.78	14.9	14.4	26.2	5.20	65.2	1.31	
			+0.147	+0.155	+0.410	+0.287	+0.424	+0.735	-0.073	-0.073
		Correlation	±.097	±.097	±.082	±.091	±.081	±.081	±.081	±.081
1927	Alfalfa II and III	Correlation	1.51	1.60	4.97	3.15	5.20	16.1	16.1	.74
		r/Er	±.117	±.601	±.703	±.738	±.925	±.735	±.925	±.065
			±.098	±.063	±.056	±.045	±.014	±.045	±.045	±.099
		r/Er	1.19	9.49	14.0	16.4	65.2	16.1	16.1	.66
1928	Silage corn	Correlation	±.185	±.175	-0.119	+0.078	±.129	-0.073	+0.065	
		r/Er	±.096	±.096	±.098	±.098	±.097	±.098	±.099	
			1.92	1.81	1.21	.79	1.31	.74	.66	
		Correlation								

In Figures 2 to 4 the correlation between each of the crop yields and all of the other crop yields<sup>3</sup> is indicated in one of the panels. The crop considered as the primary variable is shown on each panel. The crop yields considered as the secondary variables are shown at the bottom of each figure. In each panel a heavy bar represents

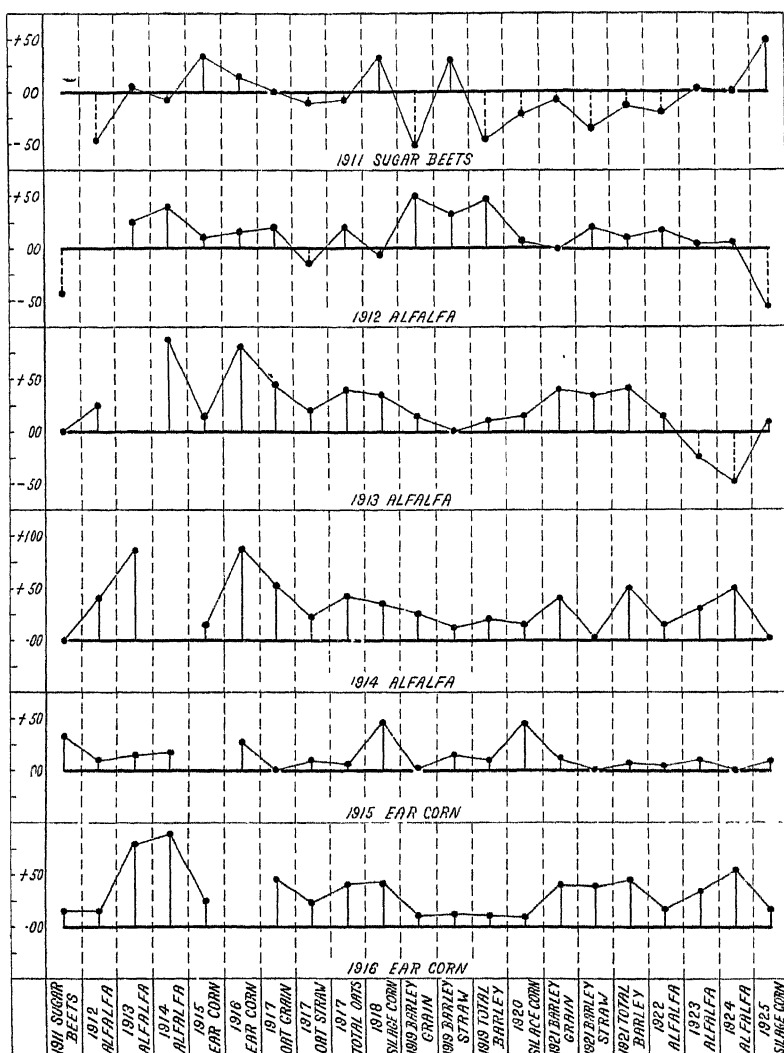


FIG. 2.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1911-1916

the zero correlation which should be found if there were no permanency of the differences in the crop-producing capacity of the several plots of the field. Positive correlations are represented by the magnitudes of the deviations above this line, and negative correlations

<sup>3</sup> In the case of the alfalfa crops the correlations for total yield only are represented, not those for individual cuttings

are represented by the deviations below this line, as shown on the scale of ordinates.

The results obtained with respect to individual crops merit detailed consideration. The correlations for the yield of sugar beets in 1911 with the 20 subsequent crop yields show large irregularities. Prac-

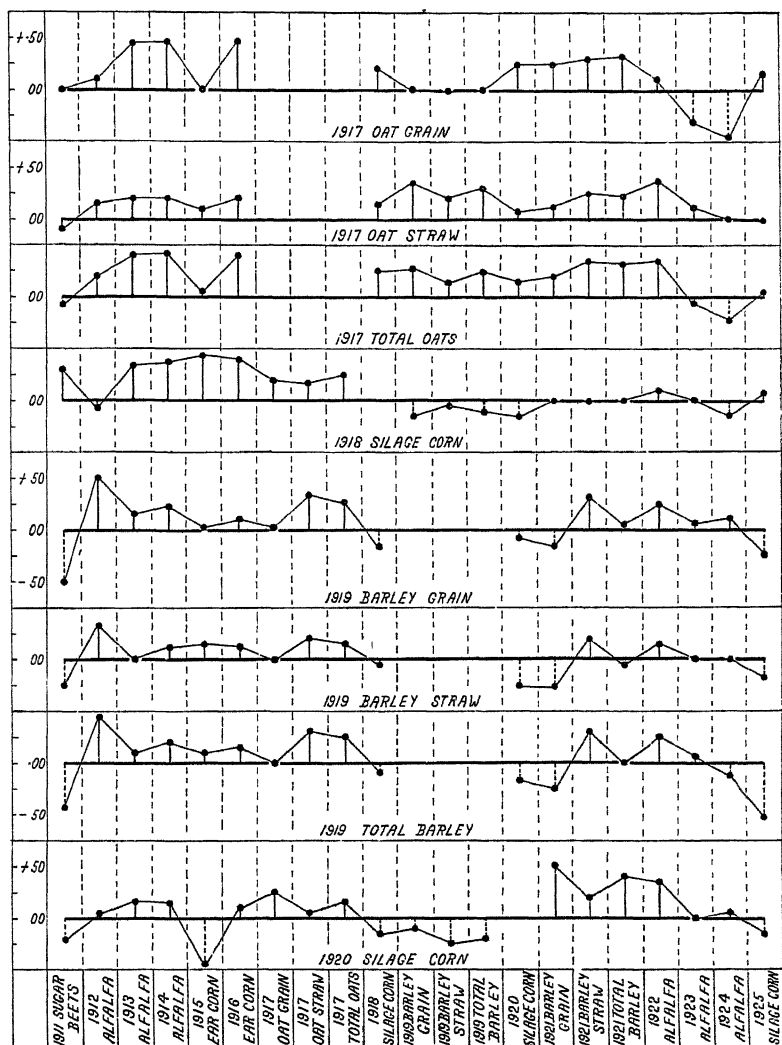


FIG. 3.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1917-1920

tically as many negative as positive correlations are seen. Taking the series as a whole, it appears that variations in the factors influencing the yield of sugar beets on these plots had no relationship to the yields of subsequent crops.

In 1912 the first of the crops of the first stand of alfalfa was harvested. For all of these crops (1912, 1913, 1914) the correlations

with antecedent crops and with practically all subsequent crops are positive in sign. Thus the original heterogeneity factors of the fields, or those introduced by differences in stand of alfalfa, persisted practically throughout the period of cultivation of this field. An exception is noted in the case of the correlation with the 1911 crop

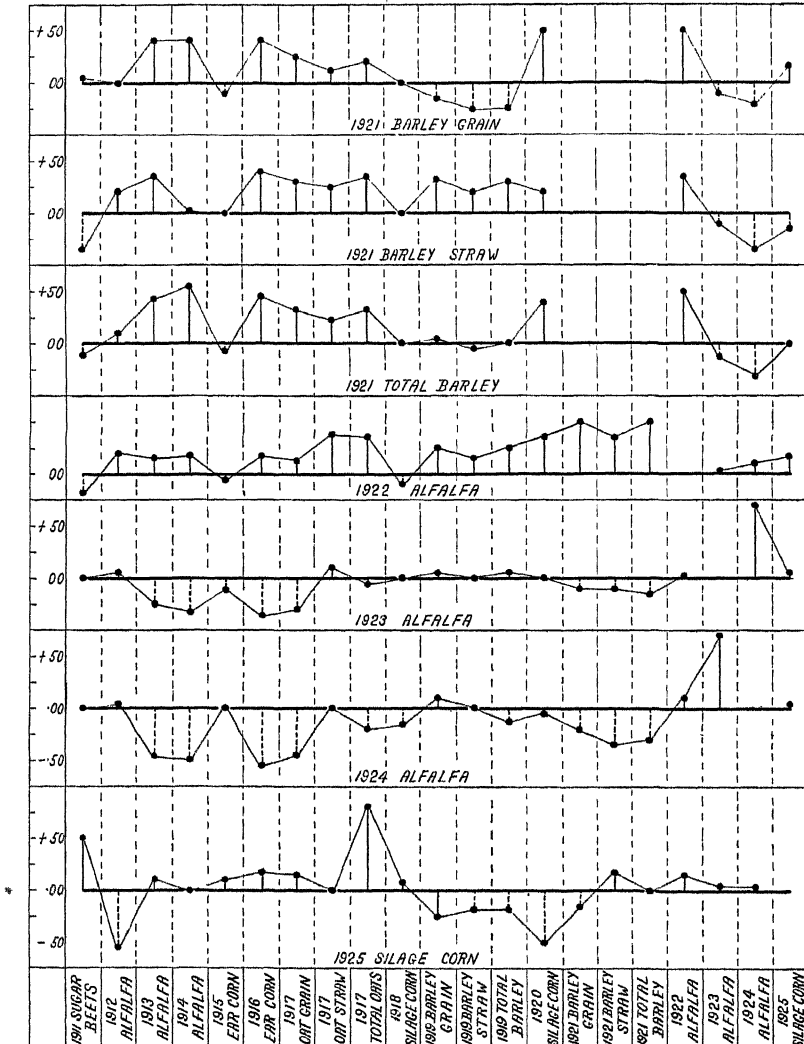


FIG. 4.—Magnitudes of correlations between the yields of various crops as grown in the uniform-cropping experiments at the Huntley Field Station, 1921-1926

of sugar beets and with the 1923 and 1924 crops of alfalfa and the 1925 crop of silage corn. The sensibly zero or even negative correlation with the crops of alfalfa grown 10 years later indicates that plots which were particularly suited for alfalfa at one period were not necessarily especially adapted to this crop at a subsequent period.

Consideration of the underlying causes of these differences in correlation is reserved until the present review of the results for the several crops is completed.

The correlations between the two crops of ear corn (1915 and 1916) and the other yields considered are generally positive. Those for 1916 are sensibly higher than those for the earlier year. This point has been considered in some detail in the former paper (7). Differences in the conditions of the soil, original or induced by agricultural technic and expressed in variations in the yield of ear corn, are therefore reflected in the yields of the other crops throughout the period of the investigation.

The three measures of oat yield in the crop grown in 1917 show a generally positive correlation with other yields except for the sugar-beet crop of 1911 and the alfalfa crops of 1923 and 1924 and the silage-corn yield of 1925. These will be considered later.

Correlations for the silage-corn yields (1918) with preceding crops are generally positive, the only exception being that for the 1912 crop of alfalfa. The relationship between the yields of this crop of silage corn and the yields of subsequent crops are, however, sensibly zero.

The three measures of the yield of barley as grown in 1919 are in general positively correlated with the yields of other crops. It is, however, interesting to note that they show a slightly negative correlation with the yield of silage corn of the preceding year (1918), of the year immediately following (1920), and of the sixth subsequent year (1925).

The 1920 crop of silage corn shows positive correlations with 12 of the other crops and negative correlations with 8 of the other crops. It shows a positive correlation with the three barley yields of the following year. This indicates that there is no necessary inverse relationship between the capacity of these plots for producing barley and corn. Turning to the three yields which furnish a measure of the capacity of these plots for barley production as grown in 1921, it is noted that in general the correlations are positive. The conspicuous exceptions are seen in the constants for alfalfa as grown in 1923 and 1924. These will be considered presently.

It should be noted that the correlations between barley as grown in 1921 and the first available test of silage corn, 1918, are sensibly zero. The correlations for the 1925 crop of silage corn are also sensibly zero. Those for the 1920 crop of silage corn, however, are more substantial positive magnitudes. The correlations with the 1915 crop of ear corn are sensibly zero, but those with the 1916 crop of ear corn are more substantial positive values. Taking these results as a whole, they indicate that there is no definitely demonstrated evidence for a negative correlation between the capacities of the plots for the production of barley and corn.

Beginning with 1922, the area was again put into alfalfa. The alfalfa yield of 1922 shows in general a positive correlation with the yields of preceding crops, but practically no correlation with the following two years' records of alfalfa or with the third subsequent year's yield of silage corn.

In the preceding investigation (7) the yields of alfalfa were found to be highly correlated with the yields of other crops. Since alfalfa occurred early in the rotation adopted in these experiments, it is

possible that differences in the yields of alfalfa influenced subsequent crops by the introduction of differences in the nitrogen content or tilth of the soil of the various plots. The present results for alfalfa are more irregular. The cutting of 1922 shows in general a positive correlation with other yields. The record for 1923 shows a sensibly zero correlation with the harvest of oat straw of 1917 to alfalfa yield of 1922. The correlation with the 1924 crop of alfalfa is high, as might be expected from the fact that both of them are influenced by the stand obtained. The correlation with the 1925 crop of silage corn is sensibly zero. The correlations with the preceding crops from the 1913 yields of alfalfa to the 1917 yields of oat grain are actually negative. Similar results are found for the 1924 crop of alfalfa. It is important to note, however, that these correlations are based on the total yields of alfalfa for these years. In both of these years one cutting (the second cutting in 1923 and the first cutting in 1924) of alfalfa was not weighed by plots. Thus, it might be suggested that because of the incompleteness of the data these yields may be inaccurate as a measure of the actual annual producing capacity of the fields. If one deals with only the first cutting in 1923, however, it is noted that only 12 of the 28 correlations are positive. If one deals with the third cutting in 1923, it is found that only 11 of the 28 correlations are positive. Similarly, if one considers only the second cutting of 1924, it appears that only 10 of the 28 correlations are positive. For the third cutting of 1924 only 8 of the 28 correlations are positive. Thus, there is clear evidence that for the individual cuttings of alfalfa there is a sensibly zero or even a negative correlation between the yields of 1923 and 1924 and those of other crops grown previously on these areas.

Turning to the final crop, silage corn as grown in 1925, it is clear that the correlations are about evenly distributed between positive and negative values.

#### DISCUSSION OF RESULTS

In an earlier investigation (?), based on the results of cultures grown from 1911 to 1919, inclusive, a high preponderance of positive correlations (many of which were clearly significant in comparison with their individual probable errors) was found between the yields of a series of plots in one year and the yields of these plots in another year. These relationships between yields were found to hold whether the crop grown was the same or different in the two years. Such results indicate clearly a relatively high permanence of the differences in the plots of the experimental field.

These findings have been confirmed by many of the constants deduced for the first time in the present investigation involving crops grown for the years 1920 to 1925. In a number of cases, however, negative correlations between the yields of crops grown on the same plots in different years have been demonstrated. This is conspicuous in the case of the correlations for alfalfa grown near the end of the experimental period.

To provide a general survey of the whole series of constants, Table 4 has been prepared. This shows the distribution of the magnitudes of the correlations between each of the crops grown and all of the other crops with the exception of crops which have a direct organic relation to the several crops listed. These have been omitted. For example, in dealing with alfalfa the correlations between the different

cuttings in one and the same year have been omitted. Similarly, the correlation between grain and straw and between either grain or straw and total yield have been omitted in the case of the cereals.

TABLE 4.—*Magnitudes of correlation coefficients measuring the relationship of crop yields on plots at Huniley, Mont., in different years*

Year	Crop	Correlation Coefficients																	
		-0.551 to -0.551	-0.551 to -0.451	-0.451 to -0.351	-0.351 to -0.251	-0.251 to -0.151	-0.151 to -0.051	-0.051 to +0.051	+0.051 to +0.151	+0.151 to +0.251	+0.251 to +0.351	+0.351 to +0.451	+0.451 to +0.551	+0.551 to +0.651	+0.651 to +0.751	+0.751 to +0.851	+0.851 to +0.951		
1911	Sugar beets.....		2	1	3	6	9	3	1	2		1							
1912	Alfalfa.....	1	1			1	3	7	9	2	4	3							
1913	Alfalfa I.....	1		1	1	2	3	5	3	1	2	3							
	Alfalfa II.....			2	3	1	2	2	3	1	2	2	3						
	Alfalfa I and II.....		2	1	2	1	2	2	4	1	4	4	2			3	1	3	
	Alfalfa I.....		2	2	1	1	1	1	2	4	3	3	2	2		2	2	1	
1914	Alfalfa II.....		2	3	1	1	2	3	3	3	3	3	1	1	1	1	1	2	
	Alfalfa I and II.....	2	1	2	1	1	1	2	2	3	1	5	2	2	2	2	2	2	
	Alfalfa III.....			1	1	2	3	2	4	1	1	5	2	2	2	2	2	2	
	Alfalfa I to III.....	1	1	2	1	1	1	6	8	1	6	4	2	1	2	2	2	2	
1915	Ear corn.....		1																
1916	do.....	2	1	2	1				5	1	4	5	1	4	1	2	3	2	
1917	(Grain.....		1	3	1	1			1	7	10	4	2						
	(Straw.....								3	4	6	3	2	2	1				
1918	(Total.....					3	3	1	4	1	2	2	2						
1919	Silage corn.....					4	6	1	2	2	6	3	2						
	(Grain.....		1			2	1	7	7	4	6	1	1						
1920	Barley.....					3	1	3	3	2	4	1							
	(Straw.....					1	3	2	4	7	5	4							
1921	(Total.....		2	1	1	4	4	2	2	6	8	1	2						
1922	Silage corn.....					4	2	3	2	4	2	4	2						
	(Grain.....					3	2	3	2	4	5	6	2						
1923	Barley.....			2	1	3	1	4	4	2	2	3	3	2					
	(Total.....			3	1	1	2	2	8	8	3	4	2						
1924	Alfalfa III.....					3	5	4	9	3	2			1					
	Alfalfa I.....					4	2	7		4	2		1						
	Alfalfa II.....		1	5		3	4												
	Alfalfa I and II.....			3	5	1	7	5	4		1								
1925	Alfalfa III.....			7	1	5	2	3	6	2									
	Alfalfa I.....			4	3	2	3	2	5	2	1	1							
	Alfalfa II.....		4	4	3	2	3	2	5	2	1	1							
	Alfalfa I and II.....		2	5	3	2	3	2	4	4				1	2				
1925	Silage corn.....		1	1		1	3	2	5	11	5			1					
Total.....		14	30	45	45	58	76	108	126	130	68	70	43	15	18	18	10		

The frequency distributions of correlation coefficients in this table show a rather wide range of both positive and negative values. These have been considered in detail on the basis of the three diagrams constituting Figures 2, 3, and 4, and nothing further need be said for the moment in regard to the relationships for the individual crops.

Taking the materials as a whole, it appears that 108 of the coefficients fall essentially in the class of zero correlations—i. e., of  $-0.05$  to  $+0.05$ .<sup>4</sup> The distribution of positive and negative coefficients on the two sides of this arbitrarily limited (zero) value is very unlike. Thus 76 constants fall between  $-0.051$  and  $-0.150$ , whereas 126 fall between the limits  $+0.051$  and  $+0.150$ . Similarly, only 58 coefficients fall between the limits of  $-0.151$  and  $-0.250$ , whereas more than twice this number (130) fall between  $+0.151$  and  $+0.250$ . Again, only 45 coefficients fall between the limits of  $-0.251$  and  $-0.350$ , whereas 68 fall between  $+0.251$  and  $+0.350$ . Finally, only 89 fall below the limit  $-0.350$ , whereas 174 fall above  $+0.350$ .

Thus the results indicate clearly the great preponderance of positive correlations for the series as a whole. Notwithstanding this fact, the figures just given show that many fairly substantial negative

<sup>4</sup> This range is probably too narrow for the zero class of coefficients based on samples of only 46 plots, but it forms a convenient unit for present purposes

coefficients occur. It is now necessary to estimate the significance of these when considered in relation to their probable errors.

The ratios of these coefficients to their probable errors, as computed by the usual formula, appear in Table 5. These ratios are arranged in class intervals of 2.50. Of these values, 299 positive coefficients fall between +0 and +2.50, whereas 169 fall between the limits of -0 and -2.50. A total of 136 coefficients fall between +2.51 and +5.00, whereas 88 coefficients fall between -2.51 and -5.00. Finally, 128 positive coefficients are more than five times as large as their probable errors, whereas only 54 negative coefficients are more than five times as large as their probable errors.

These results indicate clearly that, while there is a preponderance of positive correlations in this series and while the positive coefficients taken as a class have a greater probability of significance, certain of the negative coefficients are statistically significant in comparison with their probable errors. It appears, therefore, that in general plots which yield better in certain individual years will give better results throughout a long period of time, but that under certain conditions they may actually give yields significantly below the average.

Having demonstrated that both positive and negative correlations occur as measures of interrelationship between the crop yields of the 15 years over which these experimental cultures have extended, it still remains to analyze the series of coefficients more minutely with a view to determining, if possible, what the reasons for the apparent inconsistencies may be.

TABLE 5.—*Ratios of correlation coefficients measuring the interrelationship of yields to their probable errors for crops grown in different years on plots at Huntley, Mont.*

Year	Crop	-7.51 to -10.00	-5.01 to -7.50	-2.51 to -5.00	-0 to -2.50	+0 to +2.50	+2.51 to +5.00	+5.01 to +7.50	+7.51 to +12.50	+12.51 to +17.50	+17.51 to +25.00	+25.01 to +37.50	+37.51 to +42.50
1911	Sugar beets.....	1	2	3	13	8	2	1	-----	-----	-----	-----	-----
1912	Alfalfa.....	1	1	0	3	15	6	4	-----	-----	-----	-----	-----
	Alfalfa I.....	1	1	1	4	19	3	4	-----	-----	-----	-----	-----
1913	Alfalfa II.....	-----	-----	-----	-----	11	3	2	2	4	-----	-----	-----
	Alfalfa I and II.....	-----	2	3	1	6	5	2	1	-----	2	1	2
	Alfalfa I.....	1	1	3	2	6	5	3	1	-----	1	-----	-----
	Alfalfa II.....	1	2	3	1	7	7	1	1	-----	2	-----	-----
1914	Alfalfa I and II.....	2	1	3	1	6	4	5	-----	1	1	-----	-----
	Alfalfa III.....	-----	-----	2	6	5	6	3	3	1	1	-----	1
	Alfalfa I to III.....	1	1	4	1	7	4	1	1	1	-----	-----	2
1915	Ear corn.....	-----	1	-----	9	15	4	1	-----	-----	-----	-----	-----
1916	do.....	2	2	2	-----	9	4	3	1	2	3	1	1
	(Grain.....	-----	2	3	-----	4	6	5	7	-----	-----	-----	-----
1917	Oats.....	-----	-----	-----	4	16	8	-----	-----	-----	-----	-----	-----
	Straw.....	-----	-----	-----	7	5	14	2	-----	-----	-----	-----	-----
	Total.....	-----	-----	-----	-----	7	5	14	2	-----	-----	-----	-----
1918	Silage corn.....	-----	-----	-----	10	10	6	3	1	-----	-----	-----	-----
	(Grain.....	1	-----	1	4	13	8	1	-----	-----	-----	-----	-----
1919	Barley.....	-----	-----	3	7	17	1	-----	-----	-----	-----	-----	-----
	Straw.....	-----	-----	1	4	14	5	1	-----	-----	-----	-----	-----
	Total.....	2	1	1	4	14	5	1	-----	-----	-----	-----	-----
1920	Silage corn.....	-----	1	2	8	15	2	2	-----	-----	-----	-----	-----
	(Grain.....	-----	-----	3	6	7	7	4	1	-----	-----	-----	-----
1921	Barley.....	-----	-----	4	5	6	10	3	-----	-----	-----	-----	-----
	Straw.....	-----	-----	4	5	6	7	1	-----	-----	-----	-----	-----
	Total.....	-----	-----	4	5	6	7	1	-----	-----	-----	-----	-----
1922	Alfalfa III.....	-----	-----	3	3	18	7	1	1	-----	-----	-----	-----
	Alfalfa I.....	-----	-----	4	14	8	-----	1	1	-----	-----	-----	-----
1923	Alfalfa II.....	-----	3	7	9	6	1	-----	2	-----	-----	-----	-----
	Alfalfa I and III.....	-----	8	9	8	1	-----	1	-----	1	-----	1	-----
	Alfalfa II.....	-----	1	10	7	7	-----	-----	2	-----	1	-----	-----
1924	Alfalfa III.....	7	2	4	7	6	2	-----	-----	-----	-----	-----	-----
	Alfalfa II and III.....	2	6	4	7	6	-----	1	2	-----	-----	-----	-----
1925	Silage corn.....	2	-----	1	7	19	-----	1	-----	-----	-----	-----	-----
	Total.....	24	30	88	169	299	136	64	20	18	12	8	6



The foregoing relationships as indicated by the average values of the correlations for each of the crops grown will be first considered.

In computing these averages the constants may be divided into two groups. The first comprises correlations between each crop and every other crop grown for the period 1911 to 1919, inclusive, and is therefore identical with that for the period covered by the first investigation (7). The second comprises all correlations for the period 1920 to 1925, inclusive.

The averages and the number of correlations on which these are based are given in columns 3 to 6 of Table 6. The averages for the whole period considered, 1911 to 1925, are given in columns 7 and 8.

TABLE 6.—Averages of correlations measuring the interrelationship of yields for crops grown on plots at Huntley, Mont., in different years

Year	Crop	1911-1919		1920-1925		1911-1925		1911-1921		1923-1924	
		N	r	N	r	N	r	N	r	N	r
1911	Sugar beets.....	18	-0.0772	12	-0.0286	30	-0.0578	22	-0.0927	6	-0.0013
1912	Alfalfa.....	18	+ .2421	12	+ .0282	30	+ .1566	22	+ .2170	6	+ .0499
	Alfalfa I.....	16	+ .3456	12	+ .0009	28	+ .1978	20	+ .3525	6	+ .2832
1913	Alfalfa II.....	16	+ .4026	12	- .0906	28	+ .2041	20	+ .3614	6	+ .3106
	Alfalfa I and II.....	16	+ .4412	12	- .0345	28	+ .2373	20	+ .4224	6	+ .3480
	Alfalfa I.....	14	+ .4012	12	- .0220	26	+ .2059	18	+ .4108	6	+ .4015
	Alfalfa II.....	14	+ .3542	12	- .0920	26	+ .1483	18	+ .3434	6	+ .4167
1914	Alfalfa I and II.....	14	+ .4068	12	- .0639	26	+ .1896	18	+ .4066	6	+ .4427
	Alfalfa I.....	14	+ .3661	12	- .0200	26	+ .1879	18	+ .3426	6	+ .2187
	Alfalfa I to III.....	14	+ .4276	12	- .0917	26	+ .1879	18	+ .3961	6	+ .4100
1915	Ear corn.....	18	+ .1673	12	- .0865	30	+ .0658	22	+ .1031	6	+ .0573
1916	do.....	18	+ .4863	12	- .0765	30	+ .2612	22	+ .4602	6	+ .4458
	Grain.....	16	+ .2893	12	- .0547	28	+ .1419	20	+ .2913	6	+ .3630
1917	Oats.....	16	+ .2013	12	+ .1244	28	+ .1683	20	+ .1983	6	+ .0632
	Straw.....	16	+ .2925	12	+ .0659	28	+ .1954	20	+ .2912	6	+ .1310
1918	Silage corn.....	18	+ .2259	12	- .0467	30	+ .1169	22	+ .1813	6	+ .0751
	Grain.....	16	+ .1405	12	+ .0591	28	+ .1056	20	+ .1200	6	+ .0929
1919	Barley.....	16	+ .0863	12	- .0229	28	+ .0395	20	+ .0485	6	+ .0237
	Straw.....	16	+ .1264	12	- .0629	28	+ .0453	20	+ .0950	6	+ .0593
1920	Silage corn.....	19	+ .0285	11	+ .1010	30	+ .0551	22	+ .0780	6	+ .0452
	Grain.....	19	+ .1736	9	+ .0322	28	+ .1282	20	+ .1916	6	+ .1618
1921	Barley.....	19	+ .2549	9	+ .1108	28	+ .1373	20	+ .2525	6	+ .2374
	Straw.....	19	+ .2614	9	- .0394	28	+ .1647	20	+ .2701	6	+ .2252
1922	Alfalfa III.....	19	+ .1535	11	+ .2258	30	+ .1800	23	+ .2056	-----	-----
	Alfalfa I.....	19	- .1008	9	+ .1736	28	- .0126	23	+ .0882	-----	-----
1923	Alfalfa II.....	19	- .1671	9	+ .1052	28	- .0796	23	+ .1740	-----	-----
	Alfalfa I and III.....	19	- .1584	9	+ .1718	28	- .0522	23	+ .1497	-----	-----
	Alfalfa II.....	19	- .1894	9	+ .1813	28	- .0702	23	+ .1924	-----	-----
1924	Alfalfa III.....	19	- .3065	9	+ .0125	28	- .2039	23	+ .2856	-----	-----
	Alfalfa I and III.....	19	- .2697	9	+ .1409	28	- .1377	23	+ .2645	-----	-----
1925	Silage corn.....	19	+ .0024	11	+ .0315	30	+ .0131	23	- .0021	6	+ .0425

The number of constants averaged is larger than the number of years of experimentation because of the fact that more than one yield is available for certain of the crops (small grains and alfalfa). The correlations considered, however, have not involved relationships between two different measures of one and the same crop grown in a given year. Thus, correlations between yields of grain and straw, or between grain or straw and total yield, in the cereal crop of a given year have been excluded. Similarly, correlations between the different cuttings of alfalfa in the same year have been omitted.

In considering the values in this table it may be noted that, with the exception of the 1911 tonnage of sugar beets and the 1923 and 1924 cuttings of alfalfa, all of the average correlations for the period 1911 to 1919 are positive.

Before turning to a more general discussion of these averages, it may be noted that the yields of the single crop of sugar beets grown in 1911 have shown anomalies in correlation from the first. Unfortunately, no very precise information is available concerning the exact conditions prevailing during the first year of these experiments. Prior to the growing of sugar beets in 1911 the field had not been cropped uniformly. These antecedent differences of treatment may have led to diversities in the 1911 yields, but this is not at all certain. The writers are inclined to the view that owing to necessary difficulties in initiating these experiments in 1911 the preparation and leveling of the land may not have been as well done as in later years when the details could be cared for by men more experienced in the work. In view of these uncertainties, the data for sugar beets must be essentially disregarded in the interpretation of the results of the experiments as a whole. They have been included in the averages in order to avoid introducing any question of bias in selecting the constants on which conclusions are based. The averages of the correlations of each of the yields for the 15-year period 1911 to 1925 with the yields for 1920 to 1925 (columns 5 and 6 of Table 6) are by no means so consistent as those for the period 1911 to 1919. Of the 31 averages for 1920 to 1925, 15 are positive and 16 are negative.

An examination of the averages for the whole period of 15 years (columns 7 and 8 of Table 6) furnishes a suggestion as to the possible source of the discrepancy between the first two series of averages.

The mean correlations for the period 1911 to 1925 are positive for each of the 31 crop yields considered with the exception of the one crop of sugar beets and the cuttings of alfalfa for 1923 and 1924. This suggests that the yield of alfalfa for 1923 and 1924 may be a primary source of the frequent negative values in the series of averages for the period 1920 to 1925.

Turning back to Table 4, which gives the distribution of the various interannual correlation coefficients with regard to signs for each of the crop yields, it is noted that of the 268 correlation coefficients which are more negative than  $-0.050$  (i. e., those which fall to the left of the class which is considered to represent essentially zero correlation), 101 occur with the crops of alfalfa of 1923 and 1924.

Two new series of averages, therefore, have been determined. The first represents all crop yields from the beginning of the experiment to 1921, or until the time when the area was again put into alfalfa. The second represents the average correlation of the yields of alfalfa in 1923 and 1924 with each of the other crops (excepting 1922, 1923, and 1924 alfalfa) grown during the period 1911 to 1924.

The first of these two series of averages, presented in columns 9 and 10 of Table 6, are positive values except for the sugar-beet yield of 1911 (which has proved anomalous in practically all cases), the alfalfa yields of 1923 and 1924, and the yield of silage corn in 1925, which is sensibly zero.

The second series of averages (columns 11 and 12 of Table 6) shows 19 negative averages as compared with only 5 which are positive.

In the earlier investigation (7) emphasis was placed on certain peculiarities of the correlation for alfalfa, particularly those associated with repeated cuttings and with the after effect of the alfalfa crop.

In this connection it is worth while to lay side by side (a) the correlations between the yields of alfalfa as given in the first stand (1912-1914) and the yield of subsequent crops up to the times of reseedling to alfalfa (1915-1921) and (b) the correlation between the yields of alfalfa grown in the second stand (1922-1924) and the yields of antecedent crops back to the previous stand of alfalfa (1915-1921). Since, as already noted (7), it is quite reasonable to assume that in a crop harvested more than once a year thickness of stand and variation in the size of the individual plants will have a large influence on the yields of the different plots in the same year, the averages of the correlations between the different cuttings of the same year as well as of those between single cuttings and total cuttings in the different years of the same stand have been placed beside the above averages. The results for the correlations of alfalfa yields with the yields of subsequent crops are given in Table 7. Those for the correlation of alfalfa with the yields of antecedent crops are set forth in Table 8.

TABLE 7.—*Comparison of the averages of the correlations of alfalfa in the cuttings of 1912-1914 (a) with the yields of subsequent crops of other kinds and (b) with cuttings of alfalfa in the three different years of the same stand*

[Compare Table 8]

Year	Alfalfa crops of first stand	Average correlation with—				Difference
		(a) Other crops in 1915-1921		(b) Alfalfa yields in 1912- 1914		
		<i>N</i>	<i>r</i>	<i>N</i>	<i>r</i>	
1912	One cutting.....	13	+0.1986	8	+0.3305	+0.1319
	First cutting.....	13	+.2565	6	+.6107	+.3542
1913	Second cutting.....	13	+.2802	6	+.6038	+.3236
	First and second cuttings.....	13	+.3146	6	+.7202	+.4056
	First cutting.....	13	+.3654	4	+.6660	+.3006
	Second cutting.....	13	+.2971	4	+.6293	+.3322
1914	First and second cuttings.....	13	+.3559	4	+.6988	+.3439
	Third cutting.....	13	+.3146	4	+.5243	+.2097
	First, second, and third cuttings.....	13	+.3377	4	+.7063	+.3686

TABLE 8.—*Comparison of the averages of the correlations of alfalfa in the cuttings of 1922-1924 (a) with the yields of antecedent crops of other kinds and (b) with cuttings of alfalfa in the three different years of the same stand*

[Compare Table 7]

Year	Alfalfa crops of second stand	Average correlation with—				Difference
		(a) Other crops in 1915-1921		(b) Alfalfa yields in 1922- 1924		
		N	r	N	r	
1922	One cutting.....	13	+0.2661	6	+0.0810	-0.1851
	First cutting.....	13	-.0421	4	+.3758	+.4179
1923	Third cutting.....	13	-.1052	4	+.4738	+.5790
	First and third cuttings.....	13	-.0954	4	+.4758	+.5712
	Second cutting.....	13	-.1342	4	+.5820	+.7162
1924	Third cutting.....	13	-.2031	4	+.2508	+.4539
	First, second, and third cuttings.....	13	-.1853	4	+.5405	+.7258

Table 7 shows that the averages of the correlations between the various yields for the stand of alfalfa which occupied the land from 1912 to 1914 and the yields of subsequent crops of other kinds for the period 1915 to 1921, inclusive, are without exception positive values which range from +0.20 to +0.37. These averages are much lower than those which are found for the correlations between the yields of alfalfa in the different years of this stand. The magnitudes of the differences between the correlations between the yields of alfalfa and alfalfa and between alfalfa and other crops are clearly shown by the entries in the final column.

Turning to the averages of the correlations between the yields of alfalfa for the stand of 1922 to 1924 and the yields of antecedent crops of a different kind for the period 1915 to 1921, as shown in Table 8, a very different result is noted. These averages are negative with the exception of that for the single cutting of 1922. The averages of the correlations between the various cuttings of alfalfa in the three different years of the period 1922 to 1924 are of material positive value.

The final column shows the difference between the correlations for (a) the alfalfa cuttings of the stand and the yields of other crops and for (b) the yields of alfalfa.

A comparison of the averages in these two tables shows clearly (1) that the correlations between the yields of alfalfa in the different years are high in the case of both stands, which were separated by a period of seven years, but (2) that the correlations of subsequent crops with the stand of 1912 to 1914 are on an average positive, whereas those for antecedent crops with the stand for 1922 to 1924 are on an average negative.

It is evident that something connected with the stand or rankness of growth of the alfalfa in the latter period has been of primary importance in modifying the characteristics of the plots (as measured in terms of crop yield) as they prevailed during the first 10 years of the experiments.

In connection with the problem of changes in the heterogeneity of the field the relationships between the various yields of alfalfa in the first and second periods are of particular interest.

The individual constants required can be obtained from data given in Table 3, pages 22 and 23.

The 63 correlation coefficients for the relationship between the two different stands of alfalfa designated in Table 3 are prevalingly negative in sign. Only 13 values are positive, as compared with 50 which are negative. Of these 13 positive values, 9 are found in the correlations between the yield of alfalfa in 1922 and the yields of alfalfa in 1912, 1913, and 1914. Of the 54 correlations between cuttings of 1923 and 1924 and cuttings of 1912, 1913, and 1914, only 4 are positive.

TABLE 9.—*Averages of correlations between cuttings of alfalfa in the first and the second stands*

[Original constants are given in Table 3]

Year	Alfalfa crops	N	Average correlation with alfalfa yields
<i>1922-1924</i>			
1912	One cutting.....	7	+0.0707
	First cutting.....	7	— .2342
1913	Second cutting.....	7	— .2368
	First and second cuttings.....	7	— .2762
	First cutting.....	7	— .3020
	Second cutting.....	7	— .3403
1914	First and second cuttings.....	7	— .3486
	Third cutting.....	7	— .1787
	First, second, and third cuttings.....	7	— .3249
<i>1912-1914</i>			
1922	One cutting.....	9	+0.1630
	First cutting.....	9	— .1739
1923	Third cutting.....	9	— .2839
	First and third cuttings.....	9	— .2477
	Second cutting.....	9	— .2978
1924	Third cutting.....	9	— .4413
	Second and third cuttings.....	9	— .4071

The average values of these correlations of yields of alfalfa with other yields of alfalfa are set forth in Table 9.

With the exception of the correlations based on the single cutting for 1912 and 1922, these averages are negative throughout.

It is clear, therefore, that plots which showed superiority in alfalfa-yielding capacity in 1913 and 1914 proved on an average inferior in 1923 and 1924.

The final explanation of these results, in the opinion of the writers, must await the analysis of further data of agronomic experimentation. It is proper, however, to suggest possible explanations of observed results, since such tentative interpretations may serve to guide further work.

First of all, it is conceivable that wholly accidental differences of stand in these two periods (1912-1914 and 1922-1924) might be a source of such correlations as those which have been demonstrated. Other explanations, however, seem more probable.

The space and elevation relations of the field have already been discussed. It has been shown that the field is located within a loop of the main canal of a project that carries 400 second-feet of water. The water in this canal is 5 to 6 feet above the level of the field. The land slopes to the north and west. The southeast corner of the field, the high corner, is within 100 feet of the main canal. A shallow open ditch touches the southwest corner of the field, but curves away to the northwest.

There is a layer of sandy subsoil of possibly an acre in extent but of undetermined thickness which underlies these experimental areas. There is now definite evidence of seepage from the main canal through this sandy subsoil. A number of observation wells have been sunk around the field, three near the southeast corner and five along the north border, by which the rise and fall of the underground water may be observed. These observations have been made since the spring of 1923 only. Before that time no information was avail-

able as to the fluctuations of water in the subsoil, which is probably a phenomenon of relatively recent occurrence due to erosion in the main canal. There is reason to believe that the underground water has been rising each summer in recent years higher than it did in the earlier years of the experiment. The annual fluctuation of this underground water table is approximately 6 to 9 feet. It reaches its high point in August or September and its low point in April. In recent years the subsoil has been saturated at 1 or 2 feet below the surface in the southeast corner of the field, and it is believed that the root zone has been materially restricted throughout most of the east series (Series II).

The relationship between the yields of different portions of the field may be brought out roughly by computing the relative yields of different areas of the field. Retention of the yields of the individual plots (46 in number) results in such confusing irregularities that it is necessary to reduce the number of the plots in order to eliminate in some measure these individual variations. Since there are 23 plots in each of the two series, grouping into larger plots can be made only by taking fractions of the yields of certain of the plots occupying intermediate positions. Each of the two series has been divided into four larger plots by grouping as follows:

- (1) Plots 1 to 5 plus 75 per cent of plot 6.
- (2) Plots 7 to 11 plus 25 per cent of plot 6 plus 50 per cent of plot 12.
- (3) Plots 13 to 17 plus 50 per cent of plot 12 plus 25 per cent of plot 18.
- (4) Plots 18 to 23 plus 75 per cent of plot 18.

This divides the whole field into eight larger plots of uniform size.

After obtaining the total yields for areas of quarters of each of the two sections (eighths of the entire field), the yields have been expressed as percentages of the average yields of the eight plots. Such percentages show at a glance whether the yield of the individual plots is lower (percentages less than 100) or higher (percentages over 100) than those of the field as a whole. The numerical values can best be expressed graphically as in Figures 5 and 6. In these diagrams the four groupings of plots on the two sections of the field are represented by four panels. The relative yields for Series II are represented by solid dots connected by solid lines. The relative yields of Series III are represented by circles connected by broken lines. These yields are distributed above and below the heavy transverse bars representing 100 per cent of the average yields for the areas as a whole. Dots or circles falling below the heavy line, therefore, represent lower yields than those which are normal for the field as a whole for the given crop and year. Circles or dots falling above the line indicate yields which are above the normal for the given crop. Inspection of these diagrams shows at once that the results for sugar beets in the eight larger plots are irregular. This is in accordance with other findings for sugar beets, and the point need not be considered further.

Turning to alfalfa, which has been shown to be of particular interest, it is noted that for the first group of alfalfa yields (1912-1914) the yields per plot are in general higher than the average for the field as a whole in the case of Series II. In the case of the stand for 1922 to 1924 the reverse appears to be true in three of the four comparisons of the results for Series II and III.

This result is a somewhat different expression for the negative correlation between the alfalfa yields of the first and second series as indicated above. It extends these results by indicating that whereas in the earlier crops Series II was better for alfalfa, Series III was better for alfalfa in the later period. The writers feel inclined

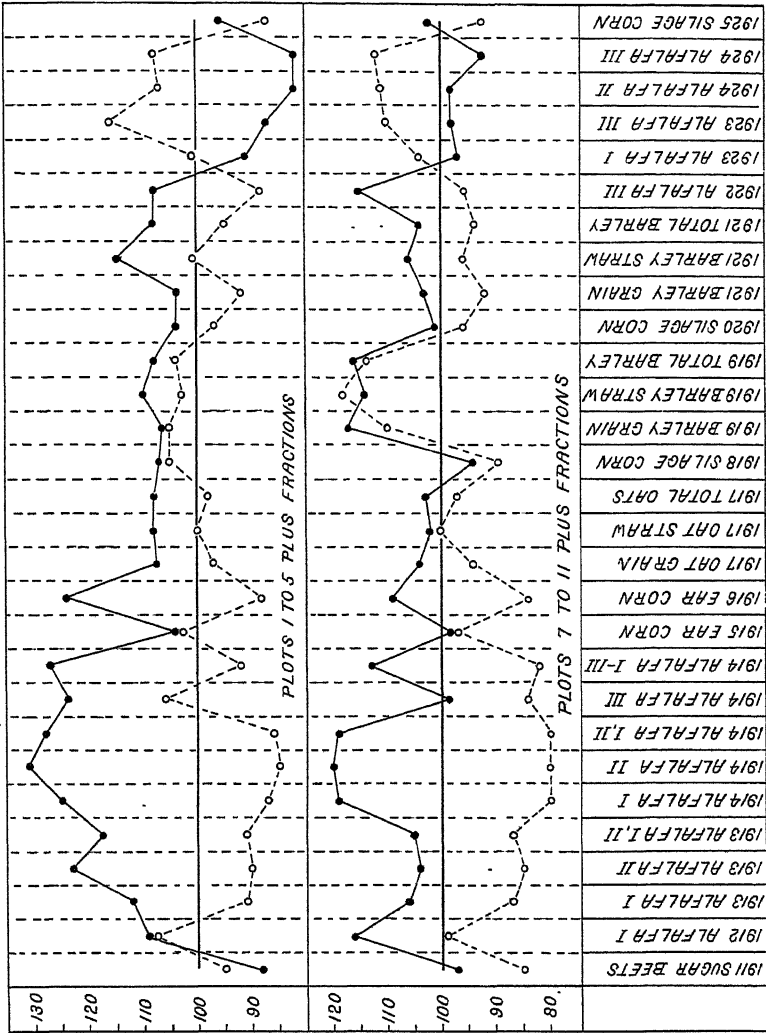


FIG 5.—Distributions of relative (percentage) yields in 8 larger plots resulting from the combination of the 23 plots of Series II and III of field B into 4 equal plots for each series. Solid dots and firm lines represent the relative yields of the 4 plots of Series II. Circles and broken lines represent the relative yields of the 4 plots of Series III. This figure represents the yields for the north half of the two series. (Compare fig. 6)

to suggest that in the earlier experiments the height of the water table had no harmful effect upon a deep-rooted crop such as alfalfa. It is quite possible that during drier periods the higher water table actually favored alfalfa growth on Series II. The higher water tables of recent years have probably had a deleterious influence,

which has been especially marked on Series II, where the water apparently comes nearer to the surface than in Series III.

Turning to relative yields of other crops for the period 1915 to 1921, it is noted that they are clustered irregularly but more closely around the 100 per cent line indicating normal yield. This suggests

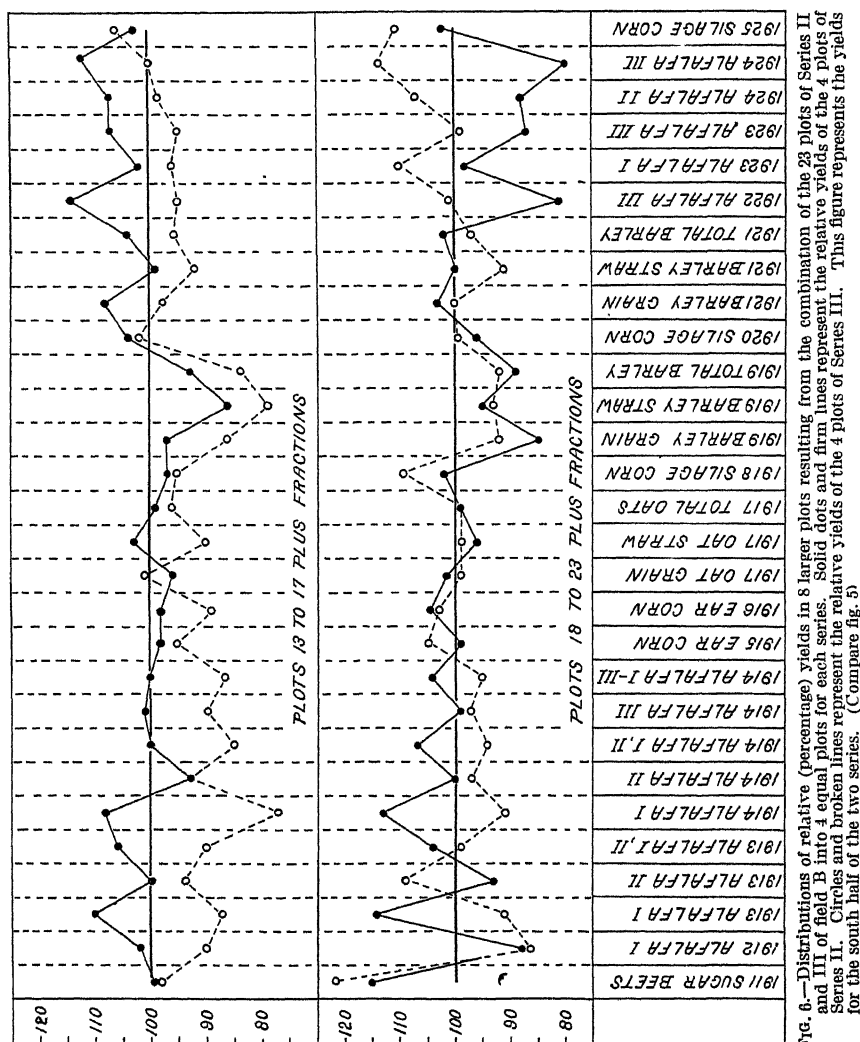


Fig. 6.—Distributions of relative (percentage) yields in 8 larger plots resulting from the combination of the 23 plots of Series II and III of field B into 4 equal plots for each series. Solid dots and firm lines represent the relative yields of the 4 plots of Series II. Circles and broken lines represent the relative yields of the 4 plots of Series III. This figure represents the yields for the south half of the two series. (Compare fig. 5.)

that these more shallow-rooted crops have not been influenced in the same way as the deeper rooted alfalfa crops by the seepage water. The first crop of alfalfa in the case of both of the two stands gives results more in accordance with those for the annual crops than do the cuttings for the second and third years. Possibly this may be due to a more superficial root system in the first year.



## SUMMARY

This paper gives the results of a biometric analysis of crop yields obtained over a period of 15 years in a uniform cropping experiment conducted by the Office of Western Irrigation Agriculture at Huntley, Mont., during the years 1911 to 1925. The records are considered with particular reference to crops grown during the period 1920 to 1925.

The results show clearly that the problem of the permanence of the heterogeneity of the agricultural experimental field is capable of treatment by the method of interannual correlation.

In general it appears that, even in small experimental tracts, there is a positive correlation between the yields of a series of plots throughout a period of years. In other words, plots which show a heavier yield one year will in general show heavier yields in other years during the period under investigation.

Under some conditions, however, negative correlations may be found—i. e., plots which produce superior yields under the conditions of one year may produce on an average inferior yields another year. In the case of the present experiments it has been suggested that fluctuations in the level of the water table in irrigated land may play a large part in determining the relative crop-yielding capacity of the different plots. A deep-rooted crop, in the present case alfalfa, seems to be more affected by these conditions than the cereals.

The importance of these results for agricultural experimentation is increased rather than diminished by the finding of significant negative as well as positive relationships between the yields of crops in different years. Both positive and negative correlations may indicate the importance of a preceding crop in determining the characteristics of an experimental field. Studies in this field have been made by Hartwell and Damon (8, 9), by Hartwell, Pember, and Merkle (10), by Garner, Lunn, and Brown (1), and by others. On the other hand, such correlations may indicate changing environmental conditions such as may be induced by variations in the level of the water table in the case of irrigation agriculture. Too great caution can not be exercised by agronomists in the selection of plots for tillage, fertilizer, or variety tests. It is clear from these results that not merely the physical and chemical characteristics of the soil but the nature of the preceding crop and changes in physical conditions from year to year must be taken into account in planning any such experiments.

Finally, it will be at once evident to agriculturists that the methods here employed may be of great value in the investigation of the after effect of the growth on land of a particular crop, or of temporarily changed physical conditions.

## LITERATURE CITED

- (1) GARNER, W. W., LUNN, W. M., and BROWN, D. E.  
1925. EFFECTS OF CROPS ON THE YIELDS OF SUCCEEDING CROPS IN THE ROTATION, WITH SPECIAL REFERENCE TO TOBACCO. *Jour. Agr. Research* 30: 1095-1132, illus.
- (2) HARRIS, J. A.  
1915. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. *Amer. Nat.* 49: 430-454.
- (3) ———  
1915. THE VALUE OF INTER-ANNUAL CORRELATIONS. *Amer. Nat.* 49: 707-712.
- (4) ———  
1920. PRACTICAL UNIVERSALITY OF FIELD HETEROGENEITY AS A FACTOR INFLUENCING PLOT YIELDS. *Jour. Agr. Research* 19: 279-314.
- (5) ———  
1926. THE SERVICE OF STATISTICAL FORMULAE IN THE ANALYSIS OF PLOT YIELDS. *Jour. Amer. Soc. Agron.* 18: 247-273, illus.
- (6) ———  
1926. THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSIOCHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. *Jour. Agr. Research* 32: 605-647, illus.
- (7) ——— and SCOFIELD, C. S.  
1920. PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD. *Jour. Agr. Research* 20: 335-356.
- (8) HARTWELL, B. L., and DAMON, S. C.  
1916. A TWENTY-YEAR COMPARISON OF DIFFERENT ROTATIONS OF CORN, POTATOES, RYE AND GRASS. *R. I. Agr. Expt. Sta. Bul.* 167, 38 p.
- (9) ——— and DAMON, S. C.  
1918. THE INFLUENCE OF CROP PLANTS ON THOSE WHICH FOLLOW. I. *R. I. Agr. Expt. Sta. Bul.* 175, 30 p., illus.
- (10) ——— PEMBER, F. R., and MERKLE, G. E.  
1919. THE INFLUENCE OF CROP PLANTS ON THOSE WHICH FOLLOW. II. *R. I. Agr. Expt. Sta. Bul.* 176, 47 p., illus.

# USE OF THE REFRACTOMETER IN THE ANALYSIS OF INDIVIDUAL SUGAR BEETS<sup>1</sup>

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## INTRODUCTION

Breeding and valuing sugar beets, especially those to be planted for the production of seed, entails the analysis of very large numbers of individuals. Criteria ordinarily used for judging the quality of commercial beets intended for the manufacture of sugar include the percentage of sucrose in the beet, the percentage of sucrose in the juice, the Brix (percentage of total soluble solids by the Brix hydrometer) of the juice, and the apparent purity (percentage of sucrose by direct polarization ÷ Brix) of the juice, but the only analytical criterion heretofore used for valuing seed beets appears to have been the percentage of sucrose in the beet. It is believed that the small size of the sample available has been the reason for limiting the analysis to this single determination. In order to minimize the mutilation of the beet and shock to it, the size of the sample removed must be restricted to the minimum quantity that will suffice for the determination of sucrose, and after the normal weight for the determination has been removed the residual sample is so small—frequently not more than 2 or 3 gm.—that the quantity of juice that may be obtained from it does not suffice for the determination of Brix or of specific gravity. The two methods available for sampling individual beets, one involving boring a hole through the beet and the other the removal of a wedge-shaped section extending from crown to tail, have been in use for many years, and various adaptations of these methods have been described by Beaudet (1),<sup>2</sup> Clark (3), Pellet (7), Pack (6), Saillard (10), Sherwood (11), and others. In all cases the sample is obtained as pulp in an exceedingly fine state of division.

At the suggestion of Dean A. Pack, associate agronomist at the Salt Lake City (Utah) field station of the Office of Sugar Plants, the writer investigated the adaptability of the refractometer for the determination of total soluble solids and apparent purity in juice from small samples removed from individual beets.

The writer has been able to find only two references relative to the subject. Komers (4) determined the percentage of sucrose in a series of samples of beet juice by means of the polariscope and the

<sup>1</sup> Received for publication Sept. 29, 1927; issued February, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 51.

percentage of total soluble solids by means of the refractometer. He subtracted the percentage of sucrose from the percentage of solids and considered the result as the percentage of nonsugar. The average of the nonsugar percentages was then used as a constant factor and subtracted from the percentage of solids by refractometer in individual samples as affording the percentage of sucrose. In a few cases the results agreed exactly with true sucrose as determined by the polariscope, but in most cases there was a plus or a minus variation, the plus variations ranging from 0.2 to 1.8 per cent and the minus ones from 0.1 to 0.9 per cent. He concluded that the method might be used for very rough approximation of sucrose and that it was superior to the ancient method of determining the comparative specific gravity of beets by floating them in salt solutions of definite specific gravity. He states that for careful and exact work the sucrose must be determined by means of the polariscope. Munerati and Mezzadrolì (5) practically repeated the investigations of Komers and arrived at the same conclusions. Neither of these investigators mentions having attempted to adapt the solids by refractometer to the calculation of apparent purity, or having compared the percentage of solids by refractometer with the percentage of solids by the Brix hydrometer.

#### SOURCES OF BEETS USED IN EXPERIMENTAL WORK

The beets used in the experimental work here reported were from the following sources:

Colorado beets: From northern Colorado, crop of 1923. Harvested in October, 1923, packed in damp earth, and stored in earthen storage cellar at just above freezing temperature until such time as refractometer investigations could be carried on (April, 1924). No doubt the long period of storage was responsible for the low percentages of sucrose. Otherwise the beets were in excellent condition.

Utah beets: From Salt Lake Valley, crop of 1924. Freshly harvested.

California beets: From the extreme southern beet-growing section, crop of 1924. Freshly harvested.

Virginia beets: From the Arlington Experiment Farm, Rosslyn, Va., crop of 1925 (experimental plots). Freshly harvested.

#### USE OF THE REFRACTOMETER

The initial investigation included the study of the effect upon the refractive index and the corresponding total soluble solids of juice expressed at different pressures and of juice expressed after different lengths of time up to 24 hours. The pulp used was in an exceedingly fine state of division, corresponding to that produced by either of the types of rasps used for sampling. After thorough mixing, 5 to 8 gm. were placed in a small piece of linen cloth, and the juice was expressed by squeezing with the fingers. The first two or three drops of juice were permitted to fall directly upon the prism of the refractometer, and the index was read. All of the juice that it was possible to express by energetic squeezing and twisting was then collected in a vessel, mixed, and the index read. The last two or three drops that it was possible to express by extreme pressing and twisting were permitted to fall directly upon the prism, and the index was read.

Portions of the samples of pulp were placed in cans provided with tight-fitting covers and stored in a refrigerator at approximately 12° C. At the expiration of the time noted in the tables the above-described procedure was repeated.

An Abbe heatable, prism refractometer was used. The light source consisted of a 50-watt mazda lamp in a box 6 by 6 by 6 inches lined with white asbestos paper and provided with a wide slot permitting light to reach the mirror of the instrument. A pane of glass was interposed between the box and the instrument in order to avoid the effect of heat from the lamp. The accuracy of the Brix hydrometers had been certified by the Bureau of Standards, and all thermometers used had been checked against thermometers the accuracy of which had been certified by the same bureau. The percentage of solids was obtained by the use of the table of Main (2) for water and solids and the correction table of Stanek (2) for temperature.

TABLE 1.—*Refractive index and total solids in juice by refractometer from stored Colorado beets*

Description of sample	Juice from fresh pulp		Juice from pulp 2 hours old		Juice from pulp 24 hours old		Percentage of total solids at 20° C. in juice from—		
	Index	Temperature, °C.	Index	Temperature, °C.	Index	Temperature, °C.	Fresh pulp	Pulp 2 hours old	Pulp 24 hours old
<b>Light pressure:</b>									
No. 1.....	1.3541	24	1.3542	24.5	1.3539	25.5	14.46	14.54	14.40
No. 2.....	1.3547	24	1.3545	24.5	1.3540	26	14.69	14.69	14.49
No. 3.....	1.3535	24.5	1.3537	24.5	1.3533	26	14.09	14.19	14.09
No. 4.....	1.3525	24.5	1.3525	24.5	1.3522	26	13.44	13.44	13.39
No. 5.....	1.3560	24.5	1.3560	24.5	1.3552	26	15.64	15.64	15.29
No. 6.....	1.3568	24.5	1.3568	24.5	1.3561	26	16.14	16.14	15.79
No. 7.....	1.3573	24.5	1.3575	24.5	1.3570	26	16.44	16.54	16.34
No. 8.....	1.3563	24.5	1.3563	24.5	1.3558	26.5	15.84	15.84	15.68
No. 9.....	1.3539	24.5	1.3539	24.5	1.3530	26.5	14.34	14.34	13.93
Average.....	1.3550		1.3550		1.3545		15.02	15.04	14.82
No. 10.....	1.3575	25					16.57		
No. 11.....	1.3558	25					15.57		
No. 12.....	1.3568	25					16.17		
No. 13.....	1.3555	25					15.37		
No. 14.....	1.3537	25					14.22		
No. 15.....	1.3555	25					15.37		
No. 16.....	1.3528	25					13.67		
No. 17.....	1.3515	25					12.82		
No. 18.....	1.3538	25					14.32		
No. 19.....	1.3543	25					14.62		
Average.....	1.3547						14.87		
No. 20.....	1.3548	25			1.3546	25	14.92		14.82
No. 21.....	1.3541	25			1.3540	25	14.52		14.42
No. 22.....	1.3548	25			1.3545	25	14.92		14.72
No. 23.....	1.3530	25			1.3530	25	13.82		13.82
No. 24.....	1.3531	25			1.3531	25	13.87		13.87
No. 25.....	1.3560	25			1.3562	25	15.67		15.82
No. 26.....	1.3540	25			1.3543	25	14.42		14.62
No. 27.....	1.3555	25			1.3552	25	15.37		15.22
No. 28.....	1.3552	25			1.3558	25	15.22		15.57
No. 29.....	1.3544	25			1.3550	25	14.67		15.07
Average.....	1.3545				1.3546		14.74		14.80

TABLE 1.—*Refractive index and total solids in juice by refractometer from stored Colorado beets—Continued*

Description of sample	Juice from fresh pulp		Juice from pulp 2 hours old		Juice from pulp 24 hours old		Percentage of total solids at 20° C. in juice from—		
	Index	Temperature, °C.	Index	Temperature, °C.	Index	Temperature, °C.	Fresh pulp	Pulp 2 hours old	Pulp 24 hours old
<b>Heavy pressure:</b>									
No. 1.....	1.3535	24	1.3535	24.5	1.3533	25.5	14.06	14.09	14.05
No. 2.....	1.3539	24	1.3539	24.5	1.3534	26	14.31	14.34	14.14
No. 3.....	1.3530	24.5	1.3530	24.5	1.3526	26	13.79	13.79	13.59
No. 4.....	1.3521	24.5	1.3520	24.5	1.3519	26	13.19	13.14	13.19
No. 5.....	1.3550	24.5	1.3550	24.5	1.3548	26	15.04	15.04	14.99
No. 6.....	1.3563	24.5	1.3562	24.5	1.3555	26	15.84	15.79	15.44
No. 7.....	1.3570	24.5	1.3570	24.5	1.3565	26	16.24	16.24	16.04
No. 8.....	1.3559	24.5	1.3559	24.5	1.3555	26.5	15.59	15.59	15.47
No. 9.....	1.3530	24.5	1.3530	24.5	1.3523	26.5	13.79	13.79	13.47
Average.....	1.3544		1.3544		1.3540		14.65	14.65	14.49
No. 10.....	1.3571	25					16.32		
No. 11.....	1.3555	25					15.37		
No. 12.....	1.3568	25					16.17		
No. 13.....	1.3553	25					15.27		
No. 14.....	1.3530	25					13.82		
No. 15.....	1.3553	25					15.27		
No. 16.....	1.3525	25					13.47		
No. 17.....	1.3511	25					12.62		
No. 18.....	1.3535	25					14.12		
No. 19.....	1.3540	25					14.42		
Average.....	1.3544						14.68		
No. 20.....	1.3543	25			1.3542	25	14.62		14.57
No. 21.....	1.3541	25			1.3539	25	14.52		14.37
No. 22.....	1.3548	25			1.3549	25	14.92		14.72
No. 23.....	1.3530	25			1.3520	25	13.82		13.17
No. 24.....	1.3531	25			1.3529	25	13.87		13.72
No. 25.....	1.3562	25			1.3562	25	15.82		15.82
No. 26.....	1.3539	25			1.3540	25	14.37		14.42
No. 27.....	1.3550	25			1.3548	25	15.07		14.92
No. 28.....	1.3552	25			1.3555	25	15.22		15.37
No. 29.....	1.3544	25			1.3544	25	14.67		14.67
Average.....	1.3544				1.3542		14.69		14.57
<b>Total mixed juice:</b>									
No. 1.....	1.3540	24	1.3540	24.5	1.3538	25.5	14.36	14.39	14.35
No. 2.....	1.3543	24	1.3543	24.5	1.3540	26	14.56	14.59	14.49
No. 3.....	1.3535	24.5	1.3535	24.5	1.3531	26	14.09	14.09	13.94
No. 4.....	1.3525	24.5	1.3525	24.5	1.3522	26	13.44	13.44	13.39
No. 5.....	1.3560	24.5	1.3560	24.5	1.3551	26	15.64	15.64	15.19
No. 6.....	1.3568	24.5	1.3568	24.5	1.3561	26	16.14	16.14	15.79
No. 7.....	1.3573	24.5	1.3575	24.5	1.3570	26	16.44	16.44	16.34
No. 8.....	1.3563	24.5	1.3563	24.5	1.3558	26.5	15.84	15.84	15.67
No. 9.....	1.3539	24.5	1.3539	24.5	1.3530	26.5	14.34	14.34	13.92
Average.....	1.3550		1.3550		1.3545		14.98	15.00	14.79
No. 10.....	1.3575	25					16.57		
No. 11.....	1.3558	25					15.57		
No. 12.....	1.3568	25					16.17		
No. 13.....	1.3555	25					15.37		
No. 14.....	1.3537	25					14.22		
No. 15.....	1.3555	25					15.37		
No. 16.....	1.3528	25					13.67		
No. 17.....	1.3515	25					12.82		
No. 18.....	1.3538	25					14.32		
No. 19.....	1.3543	25					14.62		
Average.....	1.3547						14.87		

TABLE 2.—*Refractive index and total solids in juice from fresh pulp from crown, middle, and tail sections of freshly harvested California beets*

[Each section was pulped separately and juice expressed as indicated. Determinations were made at 20° C.]

Description of sample	Light pressure		Heavy pressure		Total mixed juice	
	Index	Percentage of solids	Index	Percentage of solids	Index	Percentage of solids
No. 1 {Crown.....	1.3623	19.15	{ 1.3611	18.45	{ 1.3620	19.00
{Middle.....	1.3610	18.40		17.80		18.40
{Tail.....	1.3582	16.70		16.25		16.55
No. 2 {Crown.....	1.3630	19.60	{ 1.3625	19.30	{ 1.3629	19.50
{Middle.....	1.3635	19.90		19.50		19.75
{Tail.....	1.3640	20.20		20.05		20.20
No. 3 {Crown.....	1.3665	21.65	{ 1.3660	21.35	{ 1.3665	21.65
{Middle.....	1.3660	21.35		21.20		21.35
{Tail.....	1.3660	21.35		20.80		21.25
No. 4 {Crown.....	1.3615	18.70	{ 1.3610	18.40	{ 1.3615	18.70
{Middle.....	1.3613	18.55		18.30		18.50
{Tail.....	1.3590	17.15		17.15		17.30
No. 5 {Crown.....	1.3618	18.85	{ 1.3610	18.40	{ 1.3618	18.85
{Middle.....	1.3620	19.00		18.75		19.00
{Tail.....	1.3625	19.30		18.85		19.30
Average.....	1.36257	19.32	1.36192	18.97	1.36251	19.29
Average {Crown.....		19.59		19.18		19.54
{Middle.....		19.44		19.11		19.40
{Tail.....		18.94		18.62		18.92

Referring to the results in Tables 1 and 2, it will be noted that no difference exists between the refractive index and the total soluble solids in juice from light pressure (initial juice) and in total mixed juice, and that slightly lower results are obtained in the case of juice expressed by heavy pressure (residual juice). It is considered that the lower results in this juice were due to the effect of the so-called "colloidal water" ("colloidwasser"), which has been fully described by Rümpler (9). The quantity of this juice is so small and the difference in results so slight that its admixture with the comparatively very great quantity of total mixed juice could not result in a detectable change in the refractive index. It will also be noted that no appreciable difference exists between the results on fresh pulp and on pulp that has stood for 2 hours and for 24 hours, the very slight differences that do occur being within the operative error. In a recent communication Dean A. Pack has stated that he was able to find no difference between determinations separated by an interval of 24 to 48 hours.

It does not appear to be necessary to exert heavy pressure or to express the juice through cloth, and it is concluded that results obtained by squeezing pulp with the fingers and permitting a few drops of juice to fall directly upon the prism of the refractometer—no difficulty is encountered in obtaining a reading on this juice—afford a correct representation of the total soluble solids by refractometer in the normal juice in the pulp. It is also concluded that when the pulp is stored in a refrigerator in air-tight containers the determinations may be delayed with safety up to a period of 24 hours.

## COMPARISON OF TOTAL SOLIDS BY REFRACTOMETER AND BY BRIX HYDROMETER

One or more entire beets were pulped and the juice expressed by means of a small hand-operated screw press. After removal of occluded air and very thorough mixing the Brix reading was obtained, followed by immediate determination of the refractive index and of the percentage of sucrose by direct polarization. The results are given in Table 3.

TABLE 3.—Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources

[The percentage of sucrose given in column 2 was determined by direct polarization. In computing the approximate percentage of sucrose shown in column 11 the solids by refractometer are multiplied by the average purity by refractometer. These multipliers are 84.9 and 82 per cent (column 7) for Utah beets and for California beets, respectively. The Colorado beets were from storage; all others were freshly harvested]

Source and sample	Percentage of sucrose	Percentage of solids at 17.5° C.			Purity			Approximate nonsugars		Approximate percentage of sucrose	
		Brix	Refractometer	Variation, refractometer from Brix	Brix	Refractometer	Variation, refractometer from Brix	Brix—sucrose	Solids by refractometer—sucrose	As computed	Variation from real sucrose
1	2	3	4	5	6	7	8	9	10	11	12
Colorado beets:											
No. 1.....	8.45	15.70	15.35	-0.35	53.8	55.0	+1.2	7.25	6.90	-----	-----
No. 2.....	10.20	14.65	14.20	-0.45	69.6	71.8	+2.2	4.45	4.00	-----	-----
No. 3.....	8.60	13.20	12.60	-0.60	65.2	68.3	+3.1	4.60	4.00	-----	-----
No. 4.....	7.25	12.55	12.30	-0.25	57.8	58.9	+1.1	5.30	5.05	-----	-----
No. 5.....	10.75	14.45	14.30	-0.15	74.4	75.2	+0.8	3.70	3.55	-----	-----
No. 6.....	12.40	15.90	15.65	-0.25	78.0	79.2	+1.2	3.50	3.25	-----	-----
No. 7.....	10.30	14.35	14.10	-0.25	71.8	73.0	+1.2	4.05	3.80	-----	-----
No. 8.....	10.00	14.15	13.90	-0.25	70.7	71.9	+1.2	4.15	3.90	-----	-----
No. 9.....	7.10	11.95	11.19	-0.76	59.4	63.4	+4.0	4.85	4.09	-----	-----
No. 10.....	10.45	13.80	13.31	-0.49	75.7	78.5	+2.8	3.35	2.86	-----	-----
No. 11.....	11.40	14.45	14.16	-0.29	78.9	80.5	+1.6	3.05	2.70	-----	-----
No. 12.....	6.30	12.25	12.04	-0.21	51.4	52.3	+0.9	5.95	5.74	-----	-----
No. 13.....	9.40	13.45	12.94	-0.51	69.9	72.0	+2.1	4.05	3.54	-----	-----
No. 14.....	10.65	14.70	14.46	-0.24	72.4	73.7	+1.3	4.05	3.81	-----	-----
No. 15.....	12.90	15.95	15.81	-0.14	80.9	81.6	+0.7	3.05	2.91	-----	-----
No. 16.....	4.20	11.73	11.42	-0.31	35.8	36.8	+1.0	7.53	7.22	-----	-----
No. 17.....	7.40	12.63	12.69	+0.06	57.7	58.3	+0.6	5.43	5.29	-----	-----
No. 18.....	7.65	13.45	13.34	-0.11	56.9	57.3	+0.4	5.80	5.60	-----	-----
No. 19.....	10.70	15.30	15.24	-0.06	69.9	70.2	+0.3	4.60	4.54	-----	-----
No. 20.....	11.50	15.80	15.69	-0.11	72.8	73.3	+0.5	4.30	4.19	-----	-----
No. 21.....	11.80	15.75	15.54	-0.21	74.9	75.9	+1.0	3.95	3.79	-----	-----
No. 22.....	10.80	14.00	13.50	-0.50	74.0	74.0	0	3.80	3.79	-----	-----
No. 23.....	10.60	14.15	13.99	-0.16	74.9	75.8	+0.9	3.55	3.29	-----	-----
No. 24.....	11.30	14.85	14.59	-0.26	76.1	77.5	+1.4	3.55	3.29	-----	-----
No. 25.....	7.50	11.38	10.92	-0.46	65.9	68.7	+2.8	3.88	3.42	-----	-----
No. 26.....	8.40	11.93	11.42	-0.51	70.4	73.6	+3.2	3.53	3.02	-----	-----
No. 27.....	10.20	14.27	14.09	-0.18	71.5	72.4	+0.9	4.07	3.89	-----	-----
No. 28.....	8.00	12.45	12.12	-0.33	64.3	66.0	+1.7	4.45	4.12	-----	-----
No. 29.....	8.75	12.76	12.47	-0.29	68.6	70.2	+1.6	4.01	3.72	-----	-----
No. 30.....	7.70	12.47	12.27	-0.20	61.7	62.8	+1.1	4.77	4.57	-----	-----
No. 31.....	7.40	12.32	12.07	-0.25	60.1	61.3	+1.2	4.92	4.67	-----	-----
No. 32.....	8.70	12.88	12.67	-0.21	67.5	68.7	+1.2	4.18	3.97	-----	-----
No. 33.....	6.70	13.17	12.94	-0.23	50.9	51.8	+0.9	6.47	6.24	-----	-----
No. 34.....	9.20	13.27	13.09	-0.18	69.3	70.3	+1.0	4.07	3.80	-----	-----
No. 35.....	9.10	14.14	13.74	-0.40	64.4	66.2	+1.8	5.04	4.64	-----	-----
No. 36.....	10.10	13.99	13.74	-0.25	72.2	73.5	+1.3	3.89	3.64	-----	-----
No. 37.....	10.70	14.39	14.09	-0.30	74.4	75.9	+1.5	3.69	3.39	-----	-----
Average.....	9.31	13.77	13.49	-0.28	67.1	68.6	+1.4	4.46	4.18	-----	-----
Maximum.....	12.90	15.95	15.81	-0.14	80.9	81.6	+0.7	7.53	7.22	-----	-----
Minimum.....	4.20	11.38	10.92	-0.46	35.8	36.8	+1.0	3.05	2.76	-----	-----



TABLE 3.—*Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources—Continued*

Source and sample	Percentage of sucrose	Percentage of solids at 17.5° C.			Purity			Approximate nonsugars		Approximate percentage of sucrose	
		Brix	Refractometer	Variation, refractometer from Brix	Brix	Refractometer	Variation, refractometer from Brix	Brix—sucrose	Solids by refractometer—sucrose	As computed	Variation from real sucrose
1	2	3	4	5	6	7	8	9	10	11	12
<b>Utah beets:</b>											
No. 1.....	21.20	23.70	23.92	+0.22	89.5	88.6	-0.9	2.50	2.72	20.31	-0.89
No. 2.....	20.90	23.60	24.02	+0.42	88.6	87.0	-1.6	2.70	3.12	20.39	-0.51
No. 3.....	14.80	19.50	19.17	-0.33	75.9	77.2	+1.3	4.70	4.37	16.28	+1.48
No. 4.....	14.75	19.50	19.17	-0.33	75.6	76.9	+1.3	4.75	4.42	16.28	+1.53
No. 5.....	17.20	21.30	20.97	-0.33	80.8	82.0	+1.2	4.10	3.77	17.80	+0.60
No. 6.....	18.10	22.05	21.72	-0.33	82.1	83.3	+1.2	3.95	3.62	18.44	+0.34
No. 7.....	19.85	22.90	23.02	+0.12	86.7	86.2	-0.5	3.05	3.17	19.54	-0.31
No. 8.....	20.90	23.70	23.92	+0.22	88.2	87.4	-0.8	2.80	3.02	20.31	-0.59
No. 9.....	19.30	22.40	22.62	+0.22	86.2	85.3	-0.9	3.10	3.32	19.20	-0.10
No. 10.....	19.20	22.30	22.72	+0.42	86.1	84.5	-1.6	3.10	3.52	19.29	+0.09
No. 11.....	19.40	22.60	22.87	+0.27	85.8	84.8	-1.0	3.20	3.47	19.42	+0.02
No. 12.....	19.40	22.20	22.27	+0.07	87.4	87.1	-0.3	2.80	2.87	18.91	-0.49
No. 13.....	18.45	21.65	21.52	-0.13	85.2	85.7	+0.5	3.20	3.07	18.27	-0.18
No. 14.....	20.80	23.55	23.92	+0.37	88.3	87.0	-1.3	2.75	3.12	20.31	-0.49
No. 15.....	20.60	22.75	22.92	+0.17	90.5	89.9	-0.6	2.15	2.32	19.46	-1.14
Average.....	18.99	22.25	22.32	+0.07	85.1	84.9	-0.27	3.26	3.33	18.95	-0.04
Maximum.....	21.20	23.70	24.02	+0.42	90.5	89.0	+1.3	4.75	4.42	-----	+1.53
Minimum.....	14.75	19.50	19.17	-0.33	75.6	76.9	-1.6	2.15	2.32	-----	-1.14
<b>California beets.</b>											
No. 1.....	13.50	16.55	16.77	+0.22	81.6	80.5	-1.1	3.05	3.27	13.75	+0.25
No. 2.....	14.35	17.15	17.37	+0.22	83.7	82.6	-1.1	2.80	3.02	14.24	-0.11
No. 3.....	14.70	17.85	18.17	+0.32	82.4	80.9	-1.5	3.15	3.47	14.90	+0.20
No. 4.....	14.80	17.75	17.92	+0.17	83.4	82.6	-0.8	2.95	3.12	14.69	-0.11
No. 5.....	14.10	17.10	17.27	+0.17	82.5	81.6	-0.9	3.00	3.17	14.16	+0.06
No. 6.....	16.00	18.85	18.82	-0.03	84.9	85.0	+0.1	2.85	2.82	15.43	-0.57
No. 7.....	16.50	19.35	19.57	+0.22	85.3	84.3	-1.0	2.85	3.07	16.05	-0.45
No. 8.....	15.70	18.95	18.87	-0.08	82.8	83.2	+0.4	3.25	3.17	15.47	-0.23
No. 9.....	16.30	19.50	19.82	+0.32	83.6	82.2	-1.4	3.20	3.52	16.25	-0.05
No. 10.....	15.90	19.10	19.12	+0.02	83.2	83.2	0	3.20	3.22	15.68	-0.22
No. 11.....	14.90	17.50	17.82	+0.32	85.1	83.6	-1.5	2.60	2.92	14.61	-0.29
No. 12.....	14.90	17.45	17.62	+0.17	85.4	84.6	-0.8	2.55	2.73	14.45	-0.45
No. 13.....	15.90	18.45	18.57	+0.12	86.2	85.6	-0.6	2.55	2.67	15.23	-0.67
No. 14.....	14.00	17.00	17.27	+0.27	82.4	81.1	-1.3	3.00	3.27	14.16	+0.16
No. 15.....	15.20	17.60	17.82	+0.22	86.4	85.3	-1.1	2.40	2.62	14.61	-0.59
No. 16.....	15.25	18.00	18.07	+0.07	84.7	84.4	-0.3	2.75	2.82	14.82	-0.43
No. 17.....	15.35	18.10	18.07	-0.03	84.8	84.9	+0.1	2.75	2.72	14.82	-0.53
No. 18.....	16.45	19.55	19.77	+0.22	84.1	83.2	-0.9	3.10	3.32	16.21	-0.24
No. 19.....	13.30	16.50	16.97	+0.47	80.6	78.4	-2.2	3.20	3.67	13.92	+0.62
No. 20.....	13.45	16.55	16.72	+0.17	81.3	80.4	-0.9	3.10	3.27	13.71	+0.26
No. 21.....	12.90	16.15	16.27	+0.12	79.9	79.3	-0.6	3.25	3.37	13.34	+0.44
No. 22.....	12.65	17.45	17.62	+0.17	72.5	71.8	-0.7	4.80	4.97	14.45	+1.80
No. 23.....	13.60	17.55	17.62	+0.07	77.5	77.2	-0.3	3.95	4.02	14.45	+0.85
Average.....	14.77	17.83	18.00	+0.17	82.8	82.0	-0.8	3.06	3.23	14.76	-0.01
Maximum.....	16.50	19.55	19.82	+0.47	86.4	85.6	+0.4	4.80	4.97	-----	+1.80
Minimum.....	12.65	16.15	16.27	-0.08	72.5	71.8	-0.7	2.40	2.62	-----	-0.67
				+0.02			+0				+0.06
				-0.03			-0				-0.05

TABLE 3.—Total solids and purity in juice by Brix hydrometer and by refractometer from beets from different sources—Continued

Source and sample	Percentage of sucrose	Percentage of solids at 17.5° C.			Purity			Approximate nonsugars		Approximate percentage of sucrose	
		Brix	Refractometer	Variation, refractometer from Brix	Brix	Refractometer	Variation, refractometer from Brix	Brix—sucrose	Solids by refractometer—sucrose	As computed	Variation from real sucrose
1	2	3	4	5	6	7	8	9	10	11	12
Virginia beets:											
No. 1.....	14.70	18.30	18.40	+0.10	80.3	79.9	-0.4	3.60	3.70	-----	-----
No. 2.....	14.65	17.95	17.95	0	81.6	81.6	0	3.30	3.30	-----	-----
No. 3.....	14.90	18.85	18.60	-0.25	79.0	80.1	+1.1	3.05	3.70	-----	-----
No. 4.....	11.60	17.90	18.10	+0.20	81.6	80.7	-0.9	3.30	3.50	-----	-----
No. 5.....	15.55	19.10	19.14	+0.04	81.4	81.2	-0.2	3.55	3.59	-----	-----
No. 6.....	14.40	17.90	17.74	-0.16	80.4	81.3	+0.8	3.50	3.34	-----	-----
No. 7.....	14.50	18.20	18.30	+0.10	81.3	80.9	-0.4	3.40	3.50	-----	-----
No. 8.....	15.70	18.85	19.06	+0.21	83.3	82.4	-0.9	3.15	3.36	-----	-----
No. 9.....	16.00	19.35	19.62	+0.27	82.7	81.5	-1.2	3.35	3.62	-----	-----
No. 10.....	14.65	17.85	18.27	+0.42	82.1	80.2	-1.9	3.20	3.62	-----	-----
No. 11.....	15.50	18.80	18.90	+0.10	82.4	82.0	-0.4	3.30	3.40	-----	-----
No. 12.....	14.85	18.65	18.75	+0.10	79.6	79.2	-0.4	3.80	3.90	-----	-----
No. 13.....	14.70	18.60	18.60	0	79.0	79.0	0	3.90	3.90	-----	-----
No. 14.....	14.85	18.70	18.84	+0.14	79.4	78.8	-0.6	3.85	3.99	-----	-----
No. 15.....	13.70	17.30	17.18	-0.12	79.2	79.7	+0.5	3.60	3.48	-----	-----
No. 16.....	14.15	17.65	17.73	+0.08	80.2	79.8	-0.4	3.50	3.58	-----	-----
No. 17.....	14.00	17.65	17.58	-0.07	79.3	79.6	+0.3	3.65	3.58	-----	-----
No. 18.....	14.35	18.35	18.40	+0.05	78.2	78.0	-0.2	4.00	4.05	-----	-----
No. 19.....	14.50	17.95	17.69	-0.26	80.8	82.0	+1.2	3.45	3.19	-----	-----
No. 20.....	15.20	18.25	18.45	+0.20	83.3	82.4	-0.9	3.05	3.25	-----	-----
No. 21.....	14.25	17.80	18.12	+0.32	80.1	78.6	-1.5	3.55	3.87	-----	-----
No. 22.....	14.55	18.00	18.30	+0.30	80.8	79.5	-1.3	3.45	3.75	-----	-----
No. 23.....	14.60	18.20	18.15	-0.05	80.2	80.4	+0.2	3.60	3.55	-----	-----
No. 24.....	15.25	18.75	19.00	+0.25	81.3	80.3	-1.0	3.50	3.75	-----	-----
No. 25.....	14.20	17.65	17.80	+0.15	80.5	79.8	-0.7	3.45	3.60	-----	-----
No. 26.....	14.10	17.50	17.40	-0.10	80.6	81.0	+0.4	3.40	3.30	-----	-----
No. 27.....	14.55	18.15	18.27	+0.12	80.2	79.6	-0.6	3.60	3.72	-----	-----
No. 28.....	15.25	18.65	18.90	+0.25	81.8	80.7	-1.1	3.40	3.65	-----	-----
No. 29.....	14.40	17.85	17.84	-0.01	80.7	80.7	0	3.45	3.44	-----	-----
No. 30.....	14.70	18.20	18.30	+0.10	80.8	80.3	-0.5	3.50	3.60	-----	-----
No. 31.....	14.85	18.40	18.34	-0.06	80.7	81.0	+0.3	3.55	3.49	-----	-----
No. 32.....	14.65	18.00	18.24	+0.24	81.4	80.3	-1.1	3.35	3.59	-----	-----
No. 33.....	14.10	17.90	18.09	+0.19	78.8	77.9	-0.9	3.80	3.99	-----	-----
No. 34.....	14.50	18.70	18.84	+0.14	77.5	77.0	-0.5	4.20	4.34	-----	-----
Average.....	14.70	18.23	18.32	+0.09	80.6	80.2	-0.4	3.53	3.62	-----	-----
Maximum.....	16.00	19.35	19.62	+0.26	83.3	82.4	+1.2	4.20	4.34	-----	-----
Minimum.....	13.70	17.30	17.18	-0.12	77.5	77.0	-0.5	3.05	3.19	-----	-----
Summary:											
109 determinations—											
Average.....	13.48	17.18	17.16	-0.02	77.10	77.29	+0.14	3.70	3.68	-----	-----
Maximum.....	-----	-----	-----	+0.47	-----	-----	+1.00	-----	-----	-----	-----
Minimum.....	-----	-----	-----	-0.76	-----	-----	-2.22	-----	-----	-----	-----
72 determinations—											
Average.....	15.61	18.94	19.05	+0.11	82.25	81.75	-0.50	3.33	3.44	-----	-----
Maximum.....	-----	-----	-----	+0.47	-----	-----	+1.30	-----	-----	-----	-----
Minimum.....	-----	-----	-----	-0.33	-----	-----	-2.20	-----	-----	-----	-----

1 On freshly harvested beets only.

In the case of the stored Colorado beets (Table 3) the percentage of solids by refractometer is always lower than by Brix and the apparent purity correspondingly higher, the average variation being -0.28

per cent solids and +1.4 purity. In the case of the freshly harvested Utah, California, and Virginia beets (Table 3), the variations are irregular, but the averages are the reverse of those of the Colorado beets in that the percentage of solids by refractometer is higher than by Brix and the purity correspondingly lower. Thus, the average variation for Utah beets is +0.07 per cent and -0.27, for California beets +0.17 per cent and -0.8, and for Virginia beets +0.09 per cent and -0.4.

The averages for all samples are practically identical, the percentages of solids by refractometer being very slightly lower (-0.02 per cent) than by Brix and corresponding purity very slightly higher (+0.09), whereas, excluding the stored Colorado beets, in the averages for all freshly harvested beets the percentage of solids by refractometer is higher (+0.11 per cent) than by Brix and the corresponding purity lower (-0.5).

A survey of the literature shows that, in general, the percentage of solids by refractometer is lower than by Brix. The literature also shows that the results by refractometer more closely approach the true solids and ascribes the higher solids by Brix to the influence of the soluble nonsugars, in particular the inorganic solids, the average refractive index of which is about the same as the index for sugars, while their specific gravity is higher than that of the sugars.

Thus, Prinsen Geerligs (8) found that, in general, the calcium salts afford higher refractive indexes than sucrose, the sodium salts about equal, the potash salts lower, and that mixtures of these salts equivalent to those ordinarily occurring in sugar-plant juices afford a refractive index about equal to that of sucrose. He also found that the specific gravity of the salts is much greater than the specific gravity of sucrose, d-glucose, and d-fructose, and that while the Brix hydrometer affords correct percentages of total solids on sugar in solution it does not afford correct percentages of total solids when salts are present, the percentage of solids indicated being greater than true solids in proportion to the relative quantity of salts present.

Tolman and Smith (12) found that sucrose, d-glucose, and d-fructose have the same refractive index for all concentrations.

In the case of the stored Colorado beets, all of the results agree with the conclusions of numerous investigations in that the percentage of solids by refractometer is lower than by Brix, but in the case of the freshly harvested beets the average results are absolutely opposite in that the solids by refractometer are higher. However, great variation occurs in individual samples among the latter, the percentage of solids by refractometer varying from that by Brix by as much as +0.47 and -0.33 per cent. No doubt the stored beets must have lost considerable sucrose through respiration and sprouting. This is indicated by the high "approximate nonsugars" and consequently low ratio of sucrose to nonsugars, and it may be assumed that the higher solids by Brix were due to the high specific gravity of these nonsugars. In the freshly harvested beets the "approximate nonsugars" are much lower and are fairly consistent, and comparison of these figures with the total solids indicates that the erratic variations between solids by refractometer and by Brix are due not only to variations in the sucrose-nonsugar ratios but that they must be greatly influenced by the character of the nonsugars. However, this

investigation related merely to a comparison of results by the two methods and did not contemplate a study of the causes of variations. It is obvious that there is no constant relation between the percentage of total soluble solids and the purity as determined by refractometer and by Brix hydrometer and that the results by either method afford only a rough approximation of results by the other.

In the case of the Utah and California beets, the utilization of the results for total soluble solids by refractometer for the purpose of determining the approximate sucrose content was investigated in a manner very similar to that used by Komers (4) and Munerati and Mezzadrolì (5). The average of the purities by refractometer (sucrose÷total solids by refractometer) was used as a constant factor, and the individual total solids by refractometer were multiplied by this factor and the result considered as the approximate percentage of sucrose. These results are given in Table 3, and it will be noted that the approximate percentage of sucrose may vary from the real percentage by as much as +1.80 and -1.14 per cent. The results and conclusions agree with those of the above-mentioned investigators in that the method affords no more than an extremely rough approximation of the percentage of sucrose. High total solids by no means indicate high sucrose, and the results are so misleading that, in the opinion of the writer, the method is worthless. Sucrose must be determined by means of a saccharimeter (polariscope).

In the analysis of the small sample of pulp from an individual beet the sucrose is determined ordinarily as percentage in the beet, and it is necessary to translate this to percentage in the juice before the purity of the juice can be calculated. The formula used is—

$$\frac{\text{Percentage of sucrose in the beet}}{100 - \text{percentage of fiber in the beet}} = \text{percentage of sucrose in the juice}$$

The determination of fiber ("marc") requires so much time that it is not feasible to determine it, and incidentally in many instances the size of the sample is so small that after sucrose and total solids have been determined there does not remain sufficient material for its determination. Dry fiber in beets varies ordinarily from 4 to 5 per cent and averages about 4.5 per cent, and the use of the factor 95.5 will afford results that suffice for comparative purposes. The true fiber in situ in the beet is somewhat higher, depending upon its water of hydration, but dry-fiber figures are the ones ordinarily used. Apparent purity by refractometer is obtained by dividing the percentage of sucrose in the juice by the percentage of total soluble solids by refractometer.

TABLE 4.—*Influence of fiber upon apparent purity by refractometer*

Per centage of sucrose in beet	Per centage of total solids in juice by re- fractom- eter	Calculated percentage of sucrose in juice, on basis of fiber in beet—			Apparent purity: Sucrose in juice÷solids by re- fractometer		
		4 per cent	4 5 per cent	5 per cent	4 per cent	4.5 per cent	5 per cent
14.00	17.50	14.58	14.66	14.74	83.31	83.77	84.23
16.00	19.60	16.67	16.75	16.84	85.05	85.46	85.92
18.00	22.30	18.75	18.85	18.95	84.08	84.53	84.98
20.00	25.00	20.83	20.94	21.05	83.32	83.76	84.20
Average.....					83.04	84.38	84.83

Referring to Table 4, showing the influence of different percentages of fiber upon apparent purity, it will be seen that the variation as between 4 and 5 per cent fiber amounts to 0.9 degree. Apparent purities determined in this manner are not comparable with apparent purities as ordinarily determined by dividing the percentage of sucrose by direct polarization in the juice by Brix, as they may vary therefrom by as much as plus or minus 2 or 3 degrees, but they are comparable among themselves within a range suitable for estimating comparative purity. Indeed, there appears to be no reason why the percentage of sucrose in the beet divided by the percentage of solids by refractometer in the juice will not afford quotients that will be comparable among themselves for any given lot of beets that are in normal condition.

#### SUMMARY

Total soluble solids by refractometer in the juice from very small samples of sugar-beet pulp may be determined satisfactorily by squeezing the pulp with the fingers and permitting a few drops of juice to fall directly upon the prism of the instrument. The determination may be delayed with safety up to 24 hours if the pulp is stored in air-tight containers in a refrigerator at approximately 12° C.

The percentage of total soluble solids affords such an extremely rough and very unreliable approximation of the percentage of sucrose that it should not be used for this purpose. Sucrose should be determined by means of a saccharimeter (polariscope).

A method for determining "apparent purity by refractometer" in juice from very small samples of pulp is described. The figures are not comparable with apparent purity of juice as determined by the Brix hydrometer, as they may vary therefrom by as much as plus or minus 2 or 3 degrees, but they appear to be comparable among themselves within a comparatively small range of error. It is believed that the factor affords a valuable criterion for judging the comparative quality of individual beets which, apparently, from an analytical standpoint, heretofore have been judged on a basis of sucrose content alone.

#### LITERATURE CITED

- (1) BEAUDET, L., PELLET, H., and SAILLARD, C.  
1894. *TRAITÉ DE LA FABRICATION DU SUCRE DE BETTERAVE ET DE CANNE*. t. 2. Paris.
- (2) BROWNE, C. A.  
1912. *A HANDBOOK OF SUGAR ANALYSIS: APPENDIX*. 101 p. New York.
- (3) CLARK, W. B.  
1917. *A SAMPLING PRESS*. Jour. Indus. and Engin. Chem. 9: 788-790, illus.
- (4) KOMERS, K.  
1921. *AUSLESE VON MUTTERRÜBEN MITTELS DES REFRAKTOMETERS*. Bl. Zuckerrübenbau 28: 177-183, 194-200, 220-225, illus.
- (5) MUNERATI, O., and MEZZADROLI, G.  
1922. *L'IMPIEGO DEL REFRAATTOMETRO NELL' ESAME INDIVIDUALE DELLE BARBABIETOLE*. Staz. Sper. Agr. Ital. 55: 163-172.
- (6) PACK, D. A.  
1924. *DISC SAMPLING MACHINE*. Sugar [New York] 26: 97, illus.
- (7) PELLET, H.  
1892. *DOSAGE DU SUCRE CRISTALLISABLE DANS LA BETTERAVE*. Ann. Sci. Agron. Franç. et Étrang. 9 (1): 316-473, illus.

- 
- (8) PRINSEN GEERLIGS, H. C.  
1908. THE USE OF THE ABBE REFRACTOMETER FOR THE DETERMINATION OF DRY SUBSTANCE IN CANE JUICE AND ALL SUGAR-HOUSE PRODUCTS OF THE JAVA SUGAR INDUSTRY. *Internatl Sugar Jour.* 10: 68-81.
- (9) RÜMPLER, A.  
1898. DIE NICHTZUCKERSTOFFE DER RÜBEN. 523 p. Braunschweig.
- (10) SAILLARD, É.  
1913. BETTERAVE ET SUCRERIE DE BETTERAVES. 618 p., illus. Paris.
- (11) SHERWOOD, S. F.  
1921. SUCROSE IN SEED BEETS. *Sugar* [New York] 23: 299-300, illus.
- (12) TOLMAN, L. M., and SMITH, W. B.  
1906. ESTIMATION OF SUGARS BY MEANS OF THE REFRACTOMETER. *Jour. Amer. Chem. Soc.* 28: 1476-1482.

# FURTHER STUDIES ON THE SOIL RELATIONSHIPS OF THE MOSAIC DISEASE OF WINTER WHEAT<sup>1</sup>

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## INTRODUCTION

Simultaneously with the experiments reported in a recent paper by the writer<sup>3</sup> other studies were conducted by him on additional phases of the mosaic disease of winter wheat. It already has been emphasized that the causal agent is soil borne and that the disease has its natural origin or point of attack on the underground parts of plants in the seedling stage. However, no previous experiments have been directed toward a determination of the exact place of infection, and as a consequence it was not known whether infection occurred through the roots or the crown or both.

Similarly, no information was available on the development of the disease as influenced by growing the plants in layers of infested soil at different depths, or under different arrangement in the containers. Nor had tests been made, of the effect of dilution of the infested soil with noninfested soil, of filterability or removal of the causal agent from the infested soil, or of successively increasing the period during which the plants growing in infested soil were exposed to outdoor conditions.

It seemed desirable, therefore, to conduct experiments on the several phases enumerated. It was felt that the results would be highly important and fundamental to a proper understanding of this very complex and seemingly unusual mosaic disease. The experiments, it must be emphasized, have been very preliminary in both nature and scope, and they represent therefore only a beginning of the studies in this direction. It is recognized that the results are far from being complete and final. The data, however, certainly contribute interesting information on important relations as well as suggest a complexity of problems for subsequent research.

## METHODS AND MATERIALS

The methods involved were substantially those reported recently by the writer,<sup>4</sup> and the details may be obtained by reference to that paper. It may be added, however, that the experiments were conducted in the outdoor plots at Madison, Wis., during the winter-

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<sup>3</sup> WEBB, R. W. SOIL FACTORS INFLUENCING THE DEVELOPMENT OF THE MOSAIC DISEASE IN WINTER WHEAT. *Jour. Agr. Research* 35: 587-614, illus. 1927.

<sup>4</sup> WEBB, R. W. *Op. cit.*

wheat seasons of 1923-24 and 1924-25. All of the experiments reported have been conducted in season; that is, the grain was sown during the fall on a normal seeding date, and the plants experienced a normal or shortened period of dormancy. All precautions were taken to prevent winterkilling. Numerous punctures were made in the bottom of each container, and the containers were embedded in the ground up to the soil line over a specially prepared layer of gravel and cinders in order to facilitate underdrainage. Openings also were made in the containers above the soil line to promote free and rapid surface drainage, and the surface of the soil was sloped toward the openings. Upon the approach of low air and soil temperatures, a thin mulch was placed over the plants, and they generally passed the winter satisfactorily under these conditions.

In the first experiments only the Harvest Queen variety of wheat was tested, whereas in the later experiments both Harvest Queen and Currell varieties were used. It will be remembered that the first variety mentioned is one of the few excellent ones for expressing both the rosette and the mottling phases of the disease, while the second is one of the many excellent ones for demonstrating only the mottling symptoms. Hand-selected kernels of each variety were chosen, and the seeds were sown at the rate of 1 gram per linear foot in each of three rows in each container. In the experiments involving infested soil in cylinders of different diameters, a different method of seeding necessarily was adopted, as will be noted later. The grain was sown at a depth of 1 inch in the experiments of 1923 and 1.5 inches in those of 1924. The sowing dates in the two years were practically identical, ranging from September 28 to October 2 in 1923, and from September 30 to October 3 in 1924. Germination always was good and the seedlings emerged within the usual period.

The natural infested soil employed was the usual soil brought from Granite City, Ill. This soil is a fine sedimentary type, and is spoken of as a heavy gumbo. Fertile loam soil obtained in the vicinity of Madison, Wis., served as the noninfested soil component in the different stratification or dilution series and as a control in all the experiments.

Several different types of containers were used. For the most part, discarded metal containers from the soil-temperature tanks were employed. The diameters were 6, 8, or 10 inches, and the depths were 8 or 10 inches. Containers of uniform dimensions were used for each particular experiment, except in one case where exceptional depths were desired. Here containers 13 inches in depth were employed in conjunction with containers 8 inches in depth, the latter being used in most of the experiments. In the filtration experiments, where larger containers were desired, ordinary large ash pails were used. Small wooden flats were used in the experiments on dormancy. These flats were 12 by 14 by 8 inches.

The experiments were generally conducted in duplicate, and the results from the duplicate containers generally were very consistent. Where the infested soil was arranged as in cylinders of different diameters, the experiments were conducted in quadruplicate. The total number of plants from the duplicate containers, which survived the winter and on which these results were based, generally ranged from 60 to 100. In many cases, however, the total number was much greater than the range specified.



## STRATIFICATION OF INFESTED SOIL

In the attempt to gain information concerning the particular location on the seedling at which infection occurs, the method of soil stratification was employed.

## PROCEDURE

Layers of infested soil of different thicknesses were placed in the containers in various positions with respect to the position of the sown grain. In two experiments (A) in successive years, a layer of infested soil of varying thickness was placed in each of a series of containers, beginning at the bottom. In two similar experiments (B) the layer was from the top of the container downward. In one other experiment (C) a uniformly thin layer of infested soil was placed at different depths in the series of containers.

Experiments were conducted also (D) in which the grain was sown in infested soil arranged in the form of cylinders of different diameters, and surrounded by noninfested soil. In a complementary experiment the noninfested soil was arranged in the form of cylinders of different diameters and surrounded by infested soil.

In setting up the various stratification experiments, great care was exerted in order that the results might possess their fullest significance. Both the infested and noninfested soils were thoroughly screened and brought to excellent friability by adjustment to the proper moisture content. The lower layer of soil in each case was carefully placed and gently but firmly packed with a tamper of about the same diameter as the container. The next layer was placed and handled similarly, and the next, and so on. When the level for seeding was reached, the grain was sown and then covered with the desired thickness of soil. The layers of infested or noninfested soil thus were arranged with no possible disturbance by the addition of other layers, by the sowing of the grain, or by the covering of the grain.

Similar care was taken in the preparation of the experiments involving infested or noninfested soil in cylinders of different diameters. Noninfested soil to the depth of 4 inches was first placed in the containers. Then a hollow paraffined cylinder made of heavy paper and possessing the proper diameter was placed vertically in the center of the container, resting on the noninfested soil. Infested soil was then added either within or without the cylinder, according to the nature of the experiment. After the paper cylinder and the surrounding container had been completely filled with the proper soil, the cylinder was gently pulled out until the lower end was half an inch below the soil line. In this position the extending part of the cylinder was cut off so as to leave a 1-inch collar above the soil line and thus prevent diffusion of the two soils near the surface. These paraffined collars remained throughout the experiments.

## LAYERS OF INFESTED SOIL AT THE BOTTOM OF THE CONTAINERS

Experiment A was begun in 1923-24 and continued in 1924-25, with some expansion and other modification.

In the experiment of 1923-24 the layers of infested soil of different thicknesses were placed in the containers, beginning at the bottom.

Seven containers, 8 inches deep, were used for each variety, besides the control. The layers of infested soil ranged in thickness from 2 to 8 inches, being 2, 3, 4, 5, 6, 7, and 8 inches thick, respectively. The seeds were sown at a uniform depth of 1 inch, and the distances from the seeds to the top of the infested soil ranged by 1-inch stages from 5 to 0 inches, as noted in Table 1.

TABLE 1.—*Thickness and relative positions of the layers of infested and noninfested soil in the containers, their position in relation to the seeds sown, and the effect of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell, winter wheats, in 1923-24 and 1924-25, when the infested soil was beneath the noninfested*

[Mottling symbols: F=faint, C=conspicuous]

Season	Thickness (in inches) of layer of—		Total thickness of soil in containers (inches)	Distance from seed sown to top of layer of infested soil (inches)				Disease developing in—					
	Infested soil placed at bottom of containers	Noninfested soil placed at top of containers		Downward		Upward		Rosette (per cent)		Mottling		Currell, mottling in 1924-25	
				1923-24	1924-25	1923-24	1924-25	1923-24	1924-25	1923-24	1924-25		
1924.....	1.0	12.0	13	-----	10.5	-----	-----	0	-----	F	F	F	F
	2.0	11.0	13	-----	9.5	-----	-----	0	-----	F	F	F	F
	3.0	10.0	13	-----	8.5	-----	-----	0	-----	F	F	F	F
	4.0	9.0	13	-----	7.5	-----	-----	0	-----	F	F	F	F
	5.0	8.0	13	-----	6.5	-----	-----	0	-----	F	F	F	F
Both years.	1.0	7.0	8	-----	5.5	-----	-----	0	-----	F	F	F	F
	2.0	6.0	8	5	4.5	-----	-----	22	1.4	Present.	F	F	F
	3.0	5.0	8	4	3.5	-----	-----	33	2.5	-----	F	F	F
	4.0	4.0	8	3	2.5	-----	-----	42	4.9	-----	F	F	F
	5.0	3.0	8	2	1.5	-----	-----	42	16.9	-----	F	F	F
1924.....	6.0	2.0	8	1	.5	-----	-----	56	25.0	-----	F	F	F
Both years.	6.5	1.5	8	-----	0	-----	0	-----	28.3	-----	F	F	F
Both years.	7.0	1.0	8	0	-----	0	.5	55	70.5	Present.	C	C	C
1924.....	7.5	.5	8	-----	-----	-----	1.0	-----	80.0	-----	C	C	C
Both years.	8.0	0	8	-----	-----	1.0	1.5	90	85.1	Present.	C	C	C
	Control	8.0	8	Control.	Control.	Control.	Control.	0	0	None.	O	O	O

\* 1 stunted plant.

b 50 per cent of the plants showed conspicuous mottling.

A similar experiment was conducted in the following year, 1924-25, in which five additional containers 13 inches deep were used. The infested soil layers of different thicknesses were removed still farther from the sown grain in the deeper cans. (Fig. 1.) The thickness of the layers of infested soil was adjusted to half-inch gradations in the region of the sown grain by interpolating two more cans in the series. The methods otherwise were the same in the two years, except that the grain was sown at a depth of 1.5 inches in 1924 instead of 1 inch as in 1923.

The results of experiment A, with infested soil in the bottom of the container, are shown in Table 1 and in Figure 1. The effects on disease development are shown in Figure 1 in two ways. The percentages of rosette in the Harvest Queen variety in both years are shown by means of curves, while the intensity of mottling in both varieties is shown by a series of letters adjacent to the diagram of the containers. In these series O means no mottling; F, faint mottling; and C, conspicuous mottling. Figure 1 shows also a

schematic illustration of the series of containers used in both years, the thickness of the layers of infested soil, the distance from the wheat seeds to the top of the infested soil, and the position of the sown seed in the two years. The seedlings at a depth of 1 inch are those of 1923, and those at a depth of 1.5 inches are those of 1924.

#### EFFECT ON ROSETTE DEVELOPMENT

The curves of Figure 1 are drawn on the basis of the percentage of rosette-diseased plants as related to distance of the infested soil from the seeds. There are slight and uniform deviations in the

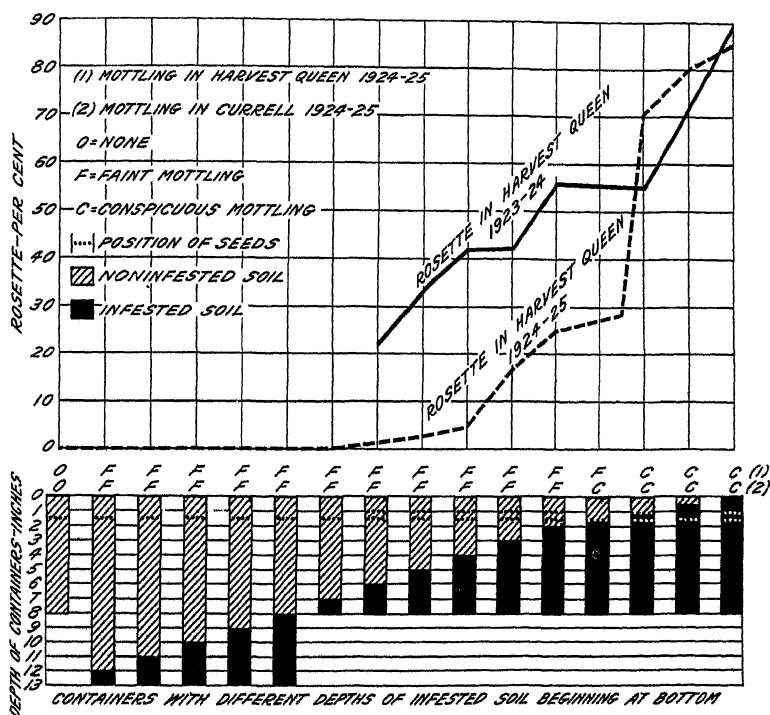


FIG. 1.—Diagram showing schematically the number and depth of containers, the position and thickness of the layers of infested soil, the position of the sown seed, the severity of mottling (by letters) of Harvest Queen in 1923-24 and of Currell in 1924-25, and the percentages of rosette (by curves) in Harvest Queen in both years with infested soil beneath the noninfested

distance between the sown grain and the infested soil of corresponding cultures in the two years. However, as the relations shown by symptom expression are very similar, and the percentages of disease in the experiment of 1923-24 were generally higher than in the corresponding experiment of 1924-25 (even though the grain was uniformly one-half inch farther from the infested soil in this case), there is justification for presenting the two curves as they are. It should be emphasized that the percentage of diseased plants at any particular point on the curves possesses relatively little importance when standing alone. It is only when each percentage is taken in conjunction with other percentages that the data assume their highest value. It is the nature and trend of the curve, therefore,

rather than any single point on the curve that really possesses significance and importance.

Examining further the results of 1923-24 in Table 1 and the corresponding curve in Figure 1, it is evident that the highest proportion of rosette-diseased plants (90 per cent) occurred in the container entirely filled, or having 8 inches of infested soil. When the layer of the infested soil was reduced to 7 inches in thickness and the grain was sown directly on the infested soil and covered with non-infested soil, the percentage diminished abruptly to 55. On reducing the thickness of the infested layer by an additional inch with a corresponding distance of 1 inch between the seeds and the infested soil, the percentage remained practically the same, namely, 56. A uniform reduction of 14 per cent in diseased plants resulted with the next two diminutions in the thickness of the layer of infested soil. In each of these containers 42 per cent of the plants showed that rosette had developed from a 5-inch and a 4-inch layer of infested soil the upper surfaces of which were 2 and 3 inches below the sown grain, respectively. A further reduction of approximately 10 per cent occurred successively in each of the next two containers of the series, the percentages of infection being 33 and 22, respectively. The latter percentage was obtained from the layer of infested soil having the least thickness (2 inches) and the greatest distance (5 inches) from the sown grain.

The effects on the rosette phase of the mosaic disease produced in the 1924-25 experiment also are tabulated in Table 1 and shown graphically in Figure 1. They demonstrate the same general relations. Although the curves from the two experiments are very similar, the percentages of diseased plants from the thin layers of infested soil considerably removed from the sown grain generally were higher in 1923-24 than in 1924-25. With greater thickness of infested soil, extending to and above the sown grain, the percentages for 1924-25 were approximately the same as, or somewhat greater than, those for 1923-24. These inconsistencies are to be expected in field studies of this type, especially when soil moisture and soil temperature play such a tremendously important part as was shown recently by Webb.<sup>4</sup>

The important features of the development of the rosette stage, however, are very consistent, despite these yearly variations. Again, the highest percentages of diseased plants occurred in the containers entirely filled with infested soil. There is a small but consistent decrease in the percentage of infection as the layer of infested soil above the seeds is diminished in thickness. Again, too, a sharp drop in the curve occurs when the grain is sown directly on the upper surface of the infested layer, with only the noninfested soil above the sown grain. The reduction was 35 per cent in 1923-24 and 57 per cent in 1924-25, and these values represent the maximum decline of the curve for any one uniform gradation within the experiment.

The rosette curve for 1924-25 shows that the presence of only a half-inch layer of infested soil above the grain, in addition to a 6½-inch layer beneath, produced a striking increase in percentage of rosette-diseased plants, compared with no infested soil above the seed. The plants from beneath the half-inch layer showed 70.5 per cent of rosette as compared to 28.3 per cent when sowing was on the

<sup>4</sup> WEBB, R. W. *Op. cit.*

surface of infested soil. It thus appears that considerable infection may and does take place through the crown of the seedling.

As noted, rosette occurred under the conditions of the extreme limits of the experiment in 1923-24, when 22 per cent of the plants showed disease which had developed from a 2-inch layer of infested soil placed 5 inches below the sown grain. In 1924-25 the range of the experiment was further expanded until the extreme containers involved only a 1-inch layer of infested soil placed 10.5 inches below the sown grain. Examining the curve further, it is evident that no rosette appeared in the container corresponding to the lower limit of the experiment during the previous year, nor in any of the deeper containers with infested soil at still greater distances from the seed. The rosette phase made its last appearance in the series with a 2-inch layer of infested soil placed either 4.5 or 5 inches below the sown grain.

#### EFFECT ON DEVELOPMENT OF MOTTLING

Thus far, only the effect on the rosette phase of the mosaic disease has been considered for the experiments of the two years. The relations concerning mottling, however, are equally as interesting. When the two sets of relations are taken together, they substantiate earlier conclusions, namely, that the rosette and the mottling expressions are different responses to the same causal agent and probably represent different degrees of severity.

From the mottling data for 1923-24 in Table 1 and the diagrammatic presentation in Figure 1, it is evident that mottling was shown by the Harvest Queen variety grown above all thicknesses of infested soil, including the thinnest layer, 2 inches, placed 5 inches below the sown grain. This is not surprising, however, inasmuch as 22 per cent of the plants developed rosette also under this set of conditions. The mottling data, however, reveal the fact that the relations representing the two disease expressions are different, especially in the range with the lesser thicknesses of infested soil placed at a considerable distance below the sown grain.

As was pointed out by the writer in a previous publication,<sup>4</sup> the mottling data are extremely difficult to express or represent, because the percentages of mottled plants and especially the intensity of the mottling are variable. No quantitative method of expression therefore is satisfactory, and no attempt is made to construct curves representing mottling on either a quantitative or a qualitative basis. Nevertheless, the rosette phase with decreased thicknesses of infested soil and increased distances below the sown grain diminishes considerably faster than the mottling phase. In brief, the mottled plants persist in numbers relatively greater than the rosetted plants and with relatively more intense symptoms under the conditions described.

In 1924-25 practically the same relations were obtained for the mottling of the Harvest Queen variety. The limits of the experiment were widened, and this expansion furnished a better opportunity for studying the phases in question. From these results, as shown in Table 1 and in Figure 1, it is evident that mottling was obtained in all the containers where infested soil was present. Furthermore, it is very evident that mottling may develop under conditions from which rosette does not develop.

<sup>4</sup> WEBB, R. W. Op. cit.

In the containers where the infested soil extended from the bottom of the can to above the sown grain, the mottling was very conspicuous, intense, and general. Such mottling has been listed as "conspicuous." With the elimination of infested soil above the sown grain, the seeds being sown directly on the infested soil and covered with noninfested soil, the intensity of the mottling declined abruptly and the percentage of mottled plants diminished noticeably. From this point to the limit of the experiment the mottling was very faint. While the percentages of mottled plants decreased with diminished thicknesses of infested soil placed at successively increasing distances from the sown grain, the percentages did not decline as rapidly as did those representing rosette. The most extreme condition which showed both rosette and mottling was a 2-inch layer of infested soil placed 4.5 inches below the sown grain. This agrees well with the results obtained during the previous year. Faint mottling persisted throughout the entire range of the experiments, even with lesser thicknesses of infested soil at greater distances below the grain.

The nonrosetted plants of the Harvest Queen variety generally showed mottling less intense than the rosetted plants of the same variety or than the mottled plants of the Currell variety. The fact that faint mottling occurred even when the grain was sown at a distance of 10.5 inches above a 1-inch layer of infested soil is very striking and proves more or less definitely that infection may and does take place through the roots.

In 1924-25 the Currell variety was tested simultaneously with the Harvest Queen variety under the conditions described. The results, as shown in Table 1, are very similar to those described for the Harvest Queen variety and need not be considered in detail. Several differences should be noted. Where the infested soil extended to or above the grain, only about 50 per cent of the plants of the Currell variety showed mottling, whereas the plants of the Harvest Queen variety showed mottling more or less generally. Such variations in mottling percentages, however, have been referred to in a recent paper by Webb.<sup>4</sup> The abrupt change in intensity of mottling from "conspicuous" to "faint" occurred generally under the same conditions as before, namely, where the infested soil was absent above the sown grain. In the case of Harvest Queen, the abrupt change in expression of symptoms occurred when the grain was sown directly on the infested soil, whereas in the case of Currell the change did not occur until a half-inch layer of noninfested soil intervened between the infested soil and the sown grain. In this connection, the relations for both mottling and rosette of the Harvest Queen variety were very similar. These relations were consistent for both rosette and mottling of the Harvest Queen variety during each of two years and for mottling of the Currell variety during one year. It thus appears very probable that considerable infection occurs through the crown itself, or through parts so immediately adjacent that they are not detectable by this method of experimentation.

#### LAYERS OF INFESTED SOIL AT THE TOP OF THE CONTAINERS

The stratification series now to be considered (experiment B) is really the reciprocal of the stratification series previously discussed and was designed to determine the effect, if any, of various thicknesses

<sup>4</sup> WEBB, R. W. Op. cit.

of infested soil beginning at the surface and extending downward to increasing distances. The usual methods were employed. Particular care was exerted to sow the grain at the proper depth and to cover it with the proper quantity of infested or noninfested soil or both. No mixing of the two layers resulted in seeding or covering the grain.

The results of these experiments are presented in Table 2, and such relations as can be shown possible are graphically and diagrammatically presented in Figure 2.

TABLE 2.—*Thickness and relative positions of the layers of infested and noninfested soil in the containers, their position in relation to the seeds sown, and the effect of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1923-24 and 1924-25, when the infested soil was above the noninfested*

[Mottling symbols: F=faint, M=midmottling, C=conspicuous]

Season	Thickness (in inches) of layer of—		Total thickness of soil in containers (inches)	Distance from seed sown to bottom of layer of infested soil (inches)				Disease developing in—							
	Infested soil placed at top of containers	Noninfested soil placed at bottom of containers		Upward		Downward		Harvest Queen				Currell, mottling in 1924-25			
								Rosette (per cent)		Mottling					
				1923-24	1924-25	1923-24	1924-25	1923-24	1924-25	1923-24	1924-25				
1924.....	0.13	7.87	8	-----	1.37	-----	-----	-----	0	-----	0	O	b F		
Both years.	.25	7.75	8	0.75	1.25	-----	-----	18 0	0	-----	M	O	b F		
	.50	7.50	8	.50	1.00	-----	-----	28 0	2.4	M	F	F	b F		
1924.....	1.00	7.00	8	0	.50	-----	-----	39 0	8.9	M	M	M	b F		
	1.50	6.50	8	-----	0	-----	-----	-----	21.7	-----	M	M	b F		
Both years.	2.00	6.00	8	-----	-----	1.00	0.50	95 0	28.9	M	M	M	b F		
	3.00	5.00	8	-----	-----	2.00	1.50	97 0	72.4	M	M	M	b F		
1924.....	4.00	4.00	8	-----	-----	3.00	2.50	97 0	74.7	M	M	M	d C		
	8.00	0	8	-----	-----	-----	6.50	-----	85.1	-----	M	M	d C		
Both years.	Control.	8.00	8	Control.	Control.	Control.	Control.	0	0	0	O	O	C		

\* One plant somewhat stunted but not typically rosetted; no mottling present on this plant.

b Faint mottling occurred but on only a small part of the plants.

c Plants somewhat stunted but not severely rosetted.

d 50 per cent of the plants showed conspicuous mottling.

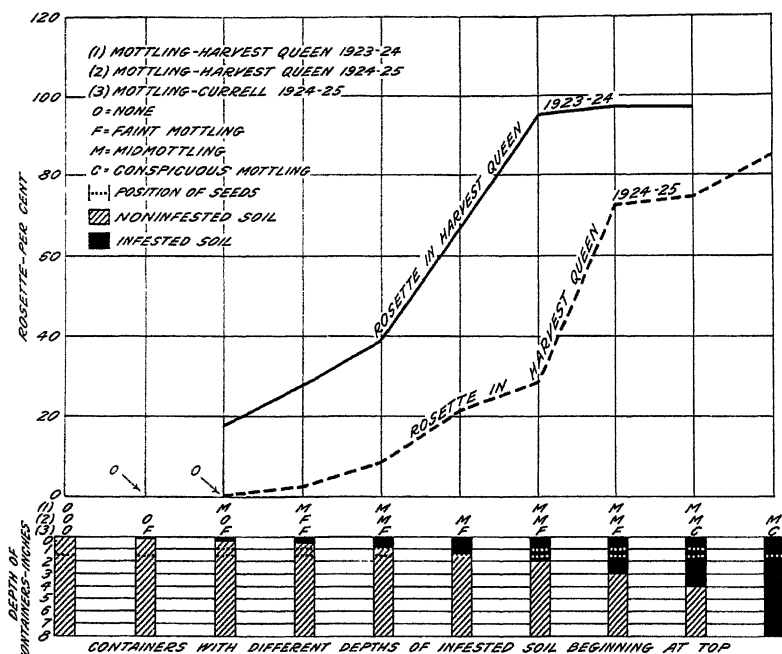
#### EFFECT ON ROSETTE DEVELOPMENT

Considering first the rosette expression in 1923-24, it is evident that rosette was obtained throughout the range of the experiment. From the test of the thinnest layer of infested soil, namely, 0.25 inch in thickness and 0.75 inch above the sown grain, 18 per cent of the plants showed definite rosette. The percentages increased to 28 with the half-inch layer and to 39 with the 1-inch layer. In the former case the lower limit of the infested soil was half an inch above the grain, and in the latter the infested soil layer rested directly on the seeds. With the 2-inch layer in which the infested soil extended 1 inch below the seeds, there was an abrupt rise in the disease curve, the value increasing from 39 to 95 per cent. With an additional 2 inches in thickness of the infested soil, the percentages remained approximately the same, namely, 97 per cent.

The effects on rosette development in 1924-25 were very consistent with those described for 1923-24. The limits of the experiment

were expanded somewhat, and finer graduations in thickness of the layers of infested soil were made in the critical zone; that is, in the region of the sown grain. Although the results were very consistent and the disease curves are very similar, the uniformly higher percentages obtained in 1923-24 as compared with those for 1924-25 are very noteworthy. The same yearly variations, however, were noted throughout all the experiments. But, as pointed out earlier in this paper, the trend of the curve is far more important than any single point on the curve.

With the two thinnest layers of infested soil, namely, 0.13 and 0.25 of an inch in thickness placed 1.37 and 1.25 inches, respectively, above the seeds, no rosette occurred. One plant in each of these





was an abrupt rise in the disease curve. The percentage was 72.4, an increase of 43.5, the greatest single change under the conditions of the experiments.

A similar relation was noted in the results from the experiments of the previous year, when the infested soil extended from the surface to a distance of 1 inch beneath the grain. It thus appears that infection can and does take place through both the crown and the roots, and these results agree well with those of the reciprocal experiment. Further, they demonstrate that relatively high percentages of rosette are possible only when the infested soil extends from 1 to 1.5 inches both above and below the sown grain. With increased thicknesses of infested soil beyond the above limits, the disease curve rises slowly.

#### EFFECT ON DEVELOPMENT OF MOTTLING

In the light of the previous results the mottling data agree very closely with what might be expected. In general, relatively higher percentages of mottling than of rosette are possible under conditions where the layers of infested soil are relatively thin and distantly removed from the seeds. In 1923-24 the mottling was of mid-intensity throughout the experiment, whereas in 1924-25 the mottling was again of medium intensity throughout except with the 0.5-inch layer of infested soil. With this, the intensity was less, and it has been graded as "faint." With further diminutions in thickness of the layers of infested soil no mottling occurred. These results are in line with the rosette data for 1924, which already have been described and contrasted with the data for 1923.

The Currell variety, when tested under similar conditions, showed mottling throughout the range of the experiment. In general, the mottling of this variety appeared more intense and conspicuous than that of the Harvest Queen variety. Several plants in each container with thicknesses of infested soil varying from 0.13 inch to 3 inches showed faint mottling. It was not until a depth of a 4-inch layer of infested soil, extending 2.5 inches below the grain, was reached that a relatively high percentage of plants showed intense mottling. With this and greater thicknesses of infested soil, 50 per cent of the plants showed conspicuous mottling. These results are very closely in line with the relations previously described in this paper.

#### HALF-INCH STRATA OF INFESTED SOIL AT DIFFERENT LEVELS IN THE CONTAINERS

The very striking results obtained from the experiments in 1923-24 suggested the importance of studying the development of the disease in relation to a uniform layer of infested soil placed at different depths with respect to the sown grain. This was done in 1924-25 (experiment C). It seemed very desirable first to employ the layer of infested soil of minimum thickness which had been found to induce a considerable production of the disease. Accordingly, a layer of infested soil of one-half inch was chosen. The experiment included such thin layers placed at half-inch intervals from the surface to 10.5 inches below the surface. As before, the experiment was conducted in duplicate, and both the Harvest Queen and Currell varieties were tested. The results are given in Table 3 and graphically shown in Figure 3, together with the schematic presentation of the position of the infested soil in the containers.

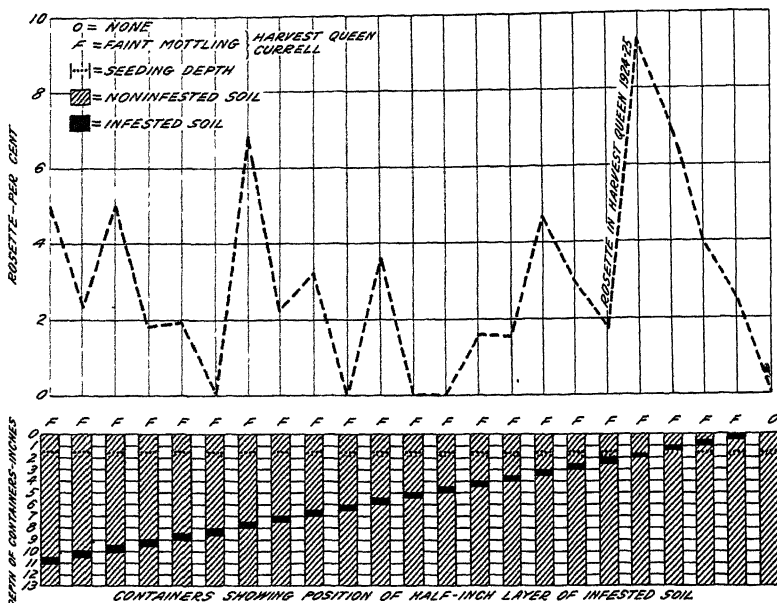


FIG. 3.—Diagram showing schematically the number and depth of the series of containers, the position of the half-inch layer of infested soil and of the seeds sown, the percentages of rosette (by curves) in the Harvest Queen variety, and the degree of mottling (F) in both the Harvest Queen and Currell varieties of winter wheat in each container, in the season of 1924-25

TABLE 3.—Position of the half-inch layers of infested soil in the 13-inch containers, with distance from the seeds sown, and effects of these factors on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1924-25

[Mottling symbol: F=faint]

Thickness of layer of noninfested soil either below or above the half-inch layer of infested soil (inches)		Distance from seeds sown to layer of infested soil (inches)		Disease developing in—		
Below	Above	Downward to top of layer	Upward to bottom of layer	Harvest Queen Rosette (per cent)	Mottling	Currell, mottling
2.0	10.5	9.0	—	5.0	F	F
2.5	10.0	8.5	—	2.3	F	F
3.0	9.5	8.0	—	5.0	F	F
3.5	9.0	7.5	—	1.8	F	F
4.0	8.5	7.0	—	1.9	F	F
4.5	8.0	6.5	—	0	F	F
5.0	7.5	6.0	—	6.7	F	F
5.5	7.0	5.5	—	2.2	F	F
6.0	6.5	5.0	—	3.2	F	F
6.5	6.0	4.5	—	0	F	F
7.0	5.5	4.0	—	3.6	F	F
7.5	5.0	3.5	—	0	F	F
8.0	4.5	3.0	—	0	F	F
8.5	4.0	2.5	—	1.6	F	F
9.0	3.5	2.0	—	1.5	F	F
9.5	3.0	1.5	—	4.7	F	F
10.0	2.5	1.0	—	2.9	F	F
10.5	2.0	.5	—	1.7	F	F
11.0	1.5	0	—	9.3	F	F
11.5	1.0	—	0	6.8	F	F
12.0	.5	—	.5	4.0	F	F
12.5	0	—	1.0	2.4	F	F

## EFFECT ON ROSETTE DEVELOPMENT

Very variable percentages of rosette developed when a thin layer of infested soil was placed at different depths in the containers. The proportion of rosette varied from 0 to 9.3 per cent, and the very irregular and inconsistent results were surprising. Certainly one or more factors other than the one under control in the experiments were influencing the development of the disease. Doubtless the layers of infested soil at different depths had decidedly different moisture contents and oxygen relations. The former is very important in the development of the disease, as shown recently by Webb.<sup>4</sup> Perhaps, also, if thicker layers of the infested soil had been placed at the different levels, more consistent effects on disease development would have resulted. It seems very evident that, under the conditions described, layers of infested soil thicker than one-half inch are essential for consistent developments of the disease. If such thin strata are employed, other influencing factors must be controlled to the highest possible degree.

Even though there was no very consistent correlation between disease development and the position of the infested soil in the experiment under consideration, several very important features are evident and substantiate previous results. In the two containers where the infested soil was directly above or below the sown grain the highest percentages of rosette resulted. When the infested soil was above the grain, rosette developed in 6.8 per cent of the plants, and where the infested soil was below the grain it developed in 9.3 per cent. It is shown again that the crown and immediately adjacent parts of the plant are very important as channels of infection. In the container where the infested soil was 6 inches below the grain, 6.7 per cent of rosette was obtained. This is the only percentage which approached those where the infested soil was in close proximity to the grain. It is surprising to note that no rosette developed in four cases, although some or all of the roots passed through the infested soil.

## EFFECT ON DEVELOPMENT OF MOTTLING

Reference is made again to Table 3 and Figure 3 for a consideration of the mottling phase. The data for both the Harvest Queen and Currell varieties are very similar and will be considered jointly. In brief, mottling occurred in all the containers having the half-inch layer of infested soil, regardless of its position relative to the seeds. The mottling in all cases was very faint, and frequently it was so indistinct as to be difficult to see, even with proper shading. Only part of the plants in each of the different containers exhibited mottling. Harvest Queen showed higher percentages than Currell.

## LAYERS OF INFESTED AND NONINFESTED SOIL IN CYLINDRICAL FORM

In the endeavor to throw further light on the location of the point of seedling infection, a simple experiment (D) was conducted with infested soil arranged in the form of cylinders of different diameters placed in the center of the containers and surrounded by noninfested soil. The procedure followed has been considered more in detail in the description of methods. Harvest Queen wheat was sown at a

<sup>4</sup> WEBB, R. W. Op. cit.

depth of 1 inch in the center of each central cylinder of infested soil and in the surrounding noninfested soil midway between the central cylinder and the metal container.

A reciprocal experiment also was conducted with the central cylinders of various diameters filled with noninfested soil and surrounded by infested soil. The seeds were sown as in the other case. The results obtained from these experiments are shown in Table 4.

TABLE 4.—*Development of the rosette phase of the mosaic disease in Harvest Queen winter wheat when grown in cylinders of different diameters containing infested soil and surrounded by noninfested soil, and the reverse, in 1924-25*

Diameter of central cylinder	Cylinders containing infested soil surrounded by noninfested soil			Cylinders containing noninfested soil surrounded by infested soil		
	Distance from seedlings in surrounding noninfested soil to cylinder of infested soil (inches)	Development of rosette on plants (per cent)		Distance from seedlings in central noninfested soil to infested soil (inches)	Development of rosette on plants (per cent)	
		Inside cylinder of infested soil	In surrounding noninfested soil		Inside cylinder of noninfested soil	In surrounding infested soil
0.5 inch	1.40	50	3.1	0.25	100	98-100
1 inch	1.25	50	0	1.50	100	98-100
2 inches	1.00	100	15.6	1.00	100	98-100
3 inches	.75	100	* 25.0	1.50	100	98-100
6 inches		98-100				

\* Additional 9.4 per cent of the plants showed only mottling.

In the experiment wherein the infested soil is contained in the central cylinders and surrounded by noninfested soil, it is seen that 50 per cent of the plants developed rosette when growing in cylinders of infested soil 0.5 inch or 1 inch in diameter and 4 inches in depth. When grown in cylinders of infested soil from 2 to 6 inches in diameter, 100 per cent of the plants showed rosette.

When grown in the surrounding zone of noninfested soil, the plants developed rosette more or less proportionally to their nearness to the infested soil. Very little or no rosette occurred in the containers with the least infested soil. The percentages of rosetted plants, arranged in the order of increasing diameters of the infested soil and decreasing distances from it, are as follows: 3.1, 0, 15.6, and 25. It is interesting to note that, in the last container, an additional 9.4 per cent of the plants showed mottling, making a total of 34.4 per cent of the plants showing symptoms of the disease. This is the only case where mottling developed in plants not showing rosette.

In the reciprocal experiment where the infested soil was placed outside of central cylinders of different diameters containing the noninfested soil, practically 100 per cent of the plants developed disease in both kinds of soil. The data are also presented in Table 4. At first glance it appears that the percentages of diseased plants in the noninfested soil were relatively higher in this series than when infested soil was inside. An explanation is very evident. In this second series the plants in the noninfested soil were completely surrounded by infested soil and the opportunities for infection were much greater than in the former case where the infested soil occurred on only one side of the plants.

## DILUTION OF INFESTED SOIL

The dilution method has been used effectively and continually in one way or another from almost the first recognition of the mosaic diseases, as well as of the virus diseases in general, to the present time. No research concerning such problems therefore is complete without a consideration of this procedure. The challenge with reference to the mosaic disease of winter wheat was even more urgent, inasmuch as this was the first mosaic disease recognized and proved to have a definite soil relationship. Furthermore, no previous experiments in this direction had been conducted. An opportunity thus was afforded by the dilution method for the study of symptom expression of a mosaic disease induced by a soil-infesting virus.

The infested soil was diluted in various proportions with noninfested soil. On the day before seeding, each type of soil was thoroughly pulverized and screened, united in volumetric proportions, and thoroughly mixed. The seeds were sown subsequently at a depth of 1.5 inches. The results of the different experiments are assembled in Table 5, and the curves appear in Figure 4.

TABLE 5.—*Effect of diluting infested soil with successively larger proportions of noninfested soil on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen and of the mottling phase in Currell winter wheats, in 1923-24 and 1924-25*

[Mottling symbols: F=faint, M=midmottling, C=conspicuous]

Dilutions expressed in proportion of infested soil to noninfested soil		Concen- tration of in- fested soil	Disease development in—					
			Harvest Queen				Currell, mottling in 1924- 25	
			Rosette		Mottling			
			1923-24	1924-25	1923-24	1924-25		
Infested soil	Non-in- fested soil							
All.	0	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>			
		100	95-98	88.2	95-98	M	C	
3	1	75.0	47	<sup>a</sup> 80.2	48	M	C	
1	1	50.0	56	86.9	56	M	C	
1	3	25.0	26	53.4	36	M	C	
1	7	12.5	11	32.7	23	F	<sup>b</sup> F	
1	15	6.3	1	11.0	6	F	F	
1	31	3.1	-----	5.3	-----	F	F	
0	All.	Control.	0	0	0	None.	None.	

<sup>a</sup> Small bunch of escaped plants at one end of row reduced the percentage.

<sup>b</sup> 1 plant only showed intense mottling.

## EFFECT ON ROSETTE DEVELOPMENT

In the experiment of 1923-24, the usual high percentage of rosette developed in plants in the undiluted infested soil, and diminished percentages occurred with diluted infested soil. With concentrations of 75 and 50 per cent of infested soil, the percentages of rosette decreased rapidly and rather uniformly to 47 and 56 per cent, respectively. Further dilutions caused more or less consistent reductions in percentages of disease, until at the lowest concentration, 6.3 per cent of infested soil, only a trace, or about 1 per cent, of rosette occurred.

Examining the data from the same dilutions in 1924-25, it will be seen that the trend of the curve so obtained is more or less similar

to that for 1923-24, except in certain particulars. At the concentrations of infested soil represented by 100, 75, and 50 per cent, practically the same proportion of rosette developed, namely, 88.2 to 80.2 per cent. It is interesting to note that in this case the value for the 50 per cent dilution was approximately the same as for the undiluted infested soil, whereas in the previous year approximately a 40 per cent reduction in rosette was produced at this infested-soil concentration. It is also interesting to note that in both years the proportion of rosette in plants from the 50 per cent dilution of infested soil was 7 to 9 per cent higher than from those in the 75 per cent concentration. These small variations, however, may be coincidental. Nevertheless, they emphasize the importance of considering the entire curve and its characteristic trend rather than any single point on the curve. When the concentrations were 25 per cent and less of infested soil, the percentages of rosette decreased

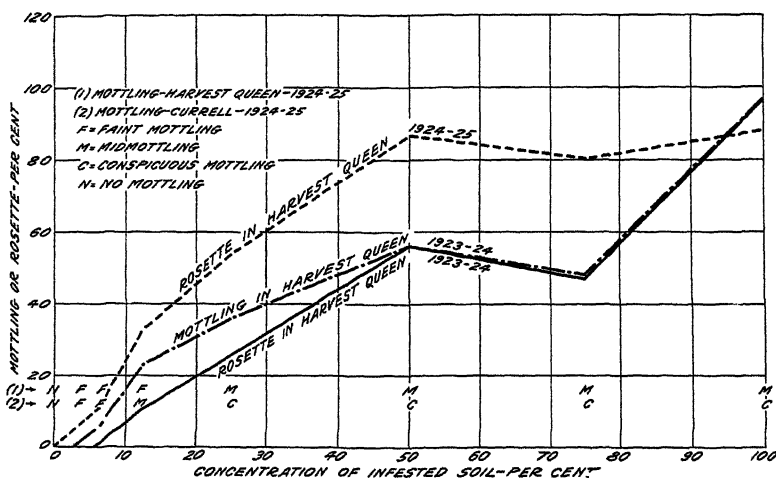


Fig. 4.—Diagram showing the effect produced on the development of the rosette and mottling phases of the mosaic disease of Harvest Queen and Currell winter wheats by diluting infested soil in different degrees by mixing with noninfested soil

rather regularly, and the part of the curve in this range is particularly similar to and consistent with that of the previous year, but the percentages in all cases are uniformly higher. The fact that 5.3 per cent of the plants developed rosette at the dilution to 3.1 per cent of infested soil is striking. However, in the previous year only a trace of rosette developed at twice this concentration.

#### EFFECT ON DEVELOPMENT OF MOTTLING

The data in regard to the development of mottling in relation to the concentration of infested soil are shown also in Table 5 and Figure 4. The percentages of mottled plants were determined in the experiments of 1923-24, and they permit definite comparison, even though this method of expression is not entirely satisfactory. The curve for mottling is identical with that for rosette until the 25 per cent concentration of infested soil is reached. Here the number of mottled plants exceeds that of rosetted plants by 10 per cent, and approximately the same relation holds for the next greater dilution,

namely, that of 12.5 per cent. With the most extensive soil dilution, the proportion of mottled plants to rosetted plants is greater even than in the cases just cited. For instance, in 12.5 per cent of infested soil the ratio is 2 to 1, whereas in 6.3 per cent the ratio is 6 to 1.

Similar relations concerning mottling were obtained during the experiments of 1924-25. Mottling developed in greater quantity and intensity in the greater dilutions than did the rosette under similar conditions. There was a distinct break in the intensity of mottling at 12.5 per cent concentration of infested soil. At concentrations of 25 per cent and above, the mottling was intense, whereas at concentrations of 12.5 per cent or less the mottling was faint.

The mottling data for the Currell variety were similar to those for Harvest Queen. The mottling was very intense and very general at all concentrations of infested soil until a concentration of only 12.5 per cent was reached. Here a sharp break occurred in both the intensity and the percentage. With a concentration of 12.5 per cent or less, the mottling became very faint and was shown by only part of the plants. These data agree well with those from Harvest Queen obtained during the same and previous years.

#### FILTRATION OF THE INFESTED SOIL

A simple experiment involving filtration methods was conducted during 1923-24. While the experiment was very preliminary in nature and scope, it was designed with the idea of throwing light on the physical nature of the infectious principle and of determining the applicability of such technic to the soil problems involved.

#### PROCEDURE

The infested soil was thoroughly pulverized and screened, placed loosely in a large Büchner funnel over double thicknesses of cheese-cloth, a considerable quantity of tap water added, and the whole filtered by suction. More water was added subsequently and drawn out. Water was added to the soil in the volumetric ratio of 2 to 1, and all possible water was drawn out of the soil. Some water, however, was retained. Considerable quantities of silt and fine particles were removed from the soil by the passage of water, and in order to remove this fraction the combined filtrates were allowed to stand overnight. The clear supernatant liquid was poured off the next day and filtered through double layers of filter paper by suction. The resulting filtrates were used as admixtures in noninfested soil.

The quantity of infested soil used in the filtration processes was twice that of the noninfested soil to which the filtrates were added. As the ratio between the water added and the infested soil was 2 to 1, the ratio between the infested soil filtered and the noninfested soil receiving the filtrates was 4 to 1. The unit quantity of noninfested soil used was half a cubic foot.

The different filtrates were applied by means of garden sprinklers to each unit quantity of noninfested soil spread out on papers in greenhouse benches. Every precaution of sterilization was taken to prevent contamination. The filtrates were added until the soil was at almost the point of saturation. The soil then was thoroughly mixed and allowed to stand. Upon standing for a day, considerable water was lost by evaporation and more of the proper filtrate was

added. These operations were repeated until the entire quantity of each filtrate had been added. The soils thus treated were stored in metal containers with metal lids and kept moist in this condition until the date of seeding, which was several weeks later.

Ash pails 11 inches in diameter and 10 inches in depth were employed as containers. The lower 5 inches of all pails was filled with the noninfested soil. The upper 4 or 5 inches in the pails was filled with the soils to which the filtrates had been added or the soils which had been filtered. In all of the previous work, infested soil 3 to 4 inches in depth has given equally as high percentages of diseased plants as greater depths of infested soil. Therefore it seemed justifiable to continue to use layers of soil of this thickness. The experiment was conducted in duplicate.

The filtrates were applied to both steam-sterilized and unsterilized noninfested soil. It seemed desirable to use sterilized soil, but in so doing there was a possibility of obtaining erroneous results, because one or more soil organisms might be instrumental in the dissemination, incubation, or inoculation of the causal agent. Thus far the available data on these points, while not conclusive, tend to be negative rather than positive. Unsterilized soil, therefore, was used to offset this possibility, but the criticism is still applicable. For instance, the infested soil came from Granite City, Ill., and contained a definite soil flora. The noninfested soil, on the other hand, came from Madison, Wis., and it also possessed a definite but probably different soil flora. All such facts should be borne in mind when studying problems of this kind.

#### EFFECT ON DEVELOPMENT OF ROSETTE AND MOTTLING

The results of the filtration experiment are shown in Table 6.

TABLE 6.—*Effect of filtering infested soil, or of adding different quantities of various filtrates to sterilized and unsterilized noninfested soil, on the development of the rosette and mottling phases of the mosaic disease in Harvest Queen winter wheat in 1924-25*

[Mottling symbols: M=midmottling, C=conspicuous]

Infestation	Soil treatment			Disease developing—	
	Sterilization	Filtration	Quantity and kind of filtrates from infested soil added	Rosette	Mottling
Noninfested	Sterilized	Control	-----	<i>Per cent</i>	
Do.	Unsterilized	do.	-----	0	Absent.
Infested	do.	Filtered once	-----	95-98	Do.
Do.	do.	Filtered twice	-----	95-98	Present
Do.	do.	Filtered three times	-----	100	Do.
Do.	do.	Not filtered	-----	95-98	Do.
Do.	do.	do.	-----	95-98	Do.
Noninfested	Sterilized	-----	7.75 gallons, cheesecloth filtrate	0	Absent.
Do.	Unsterilized	-----	do.	0	Do.
Do.	Sterilized	-----	7.75 gallons, filter-paper filtrate	0	Do.
Do.	Unsterilized	-----	do.	0	Do.
Do.	do.	-----	6.25 gallons* of silt and dregs of all filtrates.	25	M, <sup>b</sup> C, <sup>d</sup>
Do.	do.	-----	2.50 gallons of cheesecloth filtrate from infested soil that had been filtered once.	0	Absent.
Do.	do.	-----	6.50 gallons of cheesecloth filtrate from infested soil that had been filtered once.	0	Do.
Do.	do.	-----	2.75 gallons of cheesecloth filtrate from infested soil that had been filtered twice.	0	Do.

\* Fresh infested soil from field.

<sup>b</sup> Only 2 plants showed midmottling.

\* Dregs from 43 gallons of filtrate, the volume calculated as 0.268 cubic feet.

<sup>d</sup> Mottling conspicuous in all rosetted plants; a few nonrosetted plants also showed mottling.



No appreciable percentages of disease, either rosette or mottling, developed in the plants growing in soil to which filtrates from infested soil had been added. The results were negative in all cases except one, namely, where the filter-paper filtrate had been added to unsterilized soil. In this case only two plants in one of the duplicate containers showed mottling, and this would represent less than 1 per cent of the plants involved. These results are interesting in view of the fact that cheesecloth and filter-paper filtrates were added in quantities of approximately 8 gallons per 0.5 cubic foot of soil.

The silt and dregs settling out from all the filtrates were combined and the total quantity of resulting solution, 6.25 gallons, was added to a half cubic foot of soil. Calculating on a basis of the volume of particles which settled out from a known volume of filtrate upon standing overnight, it is estimated that at least 0.268 cubic foot of silt and other fine particles were added. In this soil 25 per cent of the plants developed typical rosette with very intense mottling. A few additional plants showed only mottling.

The infested soil through which large quantities of water were passed produced the disease in percentages and severity equal to that from the infested soil which had not been so treated. From the results obtained with the residues, it is evident that either relatively little or none of the virus is removed, or, if much of it is removed, it can not be detected by the methods employed. Further, the results with the filtrates indicate that little or none of the virus is removed in filtration, or, if it is removed, it soon loses its virulence. Apparently, the virus is intimately associated with the finer particles and somewhat difficult to remove from them, as is shown by the results of the filtration experiment where 25 per cent of rosette developed only upon the addition of a relatively large quantity of infested filtrate silt to the noninfested soil.

#### DISCUSSION OF RESULTS

In presenting the data, it has been necessary to consider the results in more or less detail under the different headings, and this largely eliminates the necessity for discussion at this point. However, there are several features to which reference should be made.

The quantitative determination of such disease symptoms as are reported here is a difficult problem. The rosette phase is more or less constant, and it can be expressed in terms of percentages of rosetted plants. But the mottling phase, as was pointed out by the writer in a recent paper,<sup>4</sup> is a constantly and spasmodically changing expression in regard both to percentage and to intensity.

It is strikingly evident that the disease development, especially that of the rosette phase, varied considerably in the two different years under similar experimental conditions. The results, however, were more or less consistent throughout for each year, and this feature is interesting. In general, the percentages of rosette were decidedly higher in 1923-24 than under corresponding experimental conditions in 1924-25. The seeding dates for the two years were very nearly the same, and the soil temperatures were very similar.

On examining the soil-temperature data for the several weeks subsequent to seeding in the two different years, it is evident that in

<sup>4</sup> WEBB, R. W. Op. cit.

1923 the mean weekly soil temperature was  $2^{\circ}$  to  $3^{\circ}$  C. higher and that the maximum weekly soil temperature was  $6^{\circ}$  to  $8^{\circ}$  C. higher than the corresponding values for 1924. This period is a very critical one for infection and subsequent development of the disease, as was previously shown by the writer, and the variation of a few degrees in soil temperature at this temperature range and stage of plant development may explain the results. It must be remembered, however, that soil moisture also is an important factor in this connection. All the cultures of a particular series were subjected to uniform soil temperature and soil moisture conditions. Two points are important in these relations, namely, (1) that the general nature and trend of such disease curves are more important than any single point on the curve, and (2) that too much emphasis must not be placed on the disease development under uncontrolled environmental conditions during any single year.

The results from the stratification experiments demonstrate that infection may occur through either the roots or the crown or both. Experiments of this type naturally are open to criticism, and it is not the intention of the writer to place greater value on the results than they deserve. Despite the possibilities for diffusion of soils or movement of water by either seepage or capillarity, the results are striking and suggestive.

The data at hand tend to indicate that the proportion of infection which takes place through the roots is approximately equal to, if not a little more than, that which takes place through the crown. This comparison is based chiefly on the assumption that the percentage of rosette, or symptom expression, other things being equal, is a function of the infection quantity. The following data, representing percentages of rosette, are cited as examples. In experiment A, during 1923-24, only 55 per cent of rosette developed when a layer of infested soil 6.5 inches thick was below the seeds, whereas 90 per cent developed when an additional 1-inch layer of infested soil extended above the seeds. For the similar experiment during 1924-25 the corresponding data are 28.3 and 70.5 per cent. Thus it appears that two-fifths of the rosette in 1923-24 and three-fifths in 1924-25 were caused partially or entirely by infection through the crown.

In experiment B, during 1923-24, only 39 per cent of rosette developed when a 1-inch layer of infested soil was used merely to cover the seeds, whereas 95 per cent of rosette resulted when the infested soil extended an additional inch in depth below the seeds. Corresponding data from the similar experiment during 1924-25 are 21.7 and 72.4 per cent, the latter percentage, however, resulting from a soil layer extending 1.5 inches below the seeds. Under these conditions, it appears that almost three-fifths of the rosette in 1923-24 and more than two-thirds in 1924-25 were due partially or entirely to infection through the roots. It should be stated that infested soil extending to a depth of only half an inch below the seeds increased slightly the percentage of rosette. Adding the calculated values for both crown and roots, as determined in the experiments A and B during successive years, total percentages are obtained which are surprisingly similar to the tested values for the infested-soil controls. In view of this fact, therefore, it is felt that the different calculated components representing crown and root infection, respectively, possess considerable significance.

It is of interest to note that typical rosette developed even when the top surface of the infested soil was 5 inches below the grain and that mottling developed even when the infested soil was 10.5 inches below the grain. While the cited value for rosette appears to be the limit for this expression of disease, it is very evident that the limit for the mottling phase was not reached.

In all cases where the conditions of the experiments have been such as to reduce the development of the disease, the expression of the mottling phase has persisted in higher percentages and greater intensity than the expression of the corresponding rosette phase. Only the mottling expression has appeared in certain cases. These relations further substantiate all the previous tentative conclusions, namely, that the distinctly different symptom expressions represent largely, if not entirely, different degrees of disease induced by the same causal agent.

The results obtained from dilution of the infested soil with non-infested soil are rather interesting. Experimentation of this type furnishes an excellent opportunity for study of the virus. By conducting such a dilution experiment over a period of years, the data obtained should indicate whether or not there is any change in the virus. Such an experiment would not separate the possibility of changes either in quantity or virulence or both. Nevertheless, it should yield valuable results and create suggestive leads.

The application of filtration technic to the soil problem in question introduces a complexity of problems and factors the solution of which would contribute substantially to a proper understanding of this so-called unusual mosaic disease. While the simple filtration experiment reported in this paper did not reveal any illuminating information concerning the nature of the causal agent, the results obtained under the conditions described do indicate that the causal agent is tenaciously associated with the finer soil particles. Furthermore, it would appear that the virus is more or less difficult to separate from the soil particles. However, the limited accumulation of negative results with the filtrate studies reported should not be taken as final. Additional experimentation concerning this phase is very desirable.

The infested soil, it will be remembered, is a very fine silt and belongs to the "heavy gumbo" type. The large proportion of fine particles, together with the high content of organic matter, necessarily would increase absorption and tend to make the liberation of any material, such as a virus, difficult or impossible. During the filtration process described, the soil packed and sealed in characteristic manner tightly on the cheesecloth resting in the Büchner funnel. Thus it seems very possible that the soil itself may have acted as a filter and, as such, may have retained the virus. Certainly this possibility must be considered until the results of future experimentation disprove any such relation.

If the results possess as much weight as do ordinary filtration results, it appears that the passage of large quantities of water does not remove the infectious principle. This angle of the problem has an important bearing, inasmuch as there is an open question to-day regarding the removal of the causal agent by rain water and its subsequent transportation to, and inoculation of, the soil in adjacent fields. The conditions created by heavy rains and subsequent run-

off and seepage, however, introduce differing factors. Here large quantities of water are kept in close contact with the soil particles for relatively long periods. In the filtration experiments herein reported the water was applied to the infested soil, then drawn off in a few minutes, and the operation repeated several times for each sample.

The disease developments obtained with the silt fraction of the infested soil filtrate indicate that relatively large quantities of such particles are necessary for any appreciable production of the disease. Further, the results with the infested-soil residues indicate that no detectable diminution of the disease is caused by the passage of large quantities of water, under the methods employed. Proper physical and chemical methods undoubtedly would assist materially in the successful liberation of the active agent, but there is no available evidence concerning the tolerance of the causal agent toward such treatment.

#### SUMMARY

Infection of seedlings by the causal agent of the mosaic disease of winter wheat may and does occur through either the roots or the crown or both.

The disease develops when the infested soil is either below, above, or lateral to the seeds and at a considerable distance from them.

When the infested soil occurred only below the grain, from 28 to 55 per cent of the plants showed rosette; when only above, 22 to 39 per cent; and when both below and above, usually 70 to 95 per cent. The tissues of and near the crown appear highly susceptible.

A 2-inch layer of infested soil, extending 1 inch below and 1 inch above the seeds, is sufficient to cause a relatively high percentage of rosette.

With diminishing thickness of the layer of infested soil, beginning at either the top or the bottom of the containers, the percentage and intensity of disease development is correspondingly reduced, within limits.

Five inches below the seeds was the greatest distance at which infested soil caused the rosette expression. Mottling developed at the extreme limit of the experiments, namely, with infested soil at a distance of 10.5 inches below the seeds.

A layer of infested soil 0.25 of an inch thick, placed at the surface of the container (0.75 of an inch above the seeds), caused 18 per cent of rosette in the experiments of one year and none under similar conditions in the next year.

Environmental conditions exert an important influence on the development of the disease. The general nature or trend of any disease curve, therefore, is more important than any single point on the curve.

A half-inch layer of infested soil placed at each of slightly differing successive levels in the containers produced very inconsistent results. The percentages of rosette varied from 0 to 9.3, the highest percentage occurring when the infested layer was directly beneath the seeds.

Relatively high percentages of rosette were obtained when the infested soil was diluted 50 per cent with noninfested soil. With greater dilutions, the percentages decreased more or less, and at the limit of the experiment (with only 3.1 per cent of infested soil), rosette developed to the extent of only 5.3 per cent.

Filtration of the infested soil by the passage of large quantities of water through it did not remove any detectable quantities of the

virus. In all cases, negative results were obtained when the soil filtrates were mixed with noninfested soil. Positive results were obtained only from the silt fraction removed from the filtrates. The infested soil through which water had been filtered several times produced no visible diminution in disease development.

The mottling phase persisted in relatively higher percentages and greater intensity, under the conditions described, than the rosette phase. Mottling generally was present at the limits of the experiment, whereas rosette was either absent or greatly reduced at such extremes.

Both the Harvest Queen and Currell varieties showed very similar mottling relations. In general, the percentages of mottled plants were greater in the former than in the latter.

Unmistakable symptoms of rosette were obtained when the plants were exposed to outdoor conditions from a normal seeding date (September 28) until October 31. After a longer exposure, the symptom expression was greater, both in percentage and intensity.



# SMUT SUSCEPTIBILITY OF NATURALLY RESISTANT CORN WHEN ARTIFICIALLY INOCULATED<sup>1</sup>

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## INTRODUCTION

Certain lines of corn are resistant to natural infection by smut, *Ustilago zeae*, under field conditions. This has been shown by Jones (6),<sup>3</sup> Hayes and coauthors (4), Garber and Quisenberry (3), and Immer and Christensen (5), who found differences in the reaction of selfed lines of corn and their crosses to corn smut. They conclude that these differences are heritable and that by selection in self-fertilized lines it is possible to obtain lines of corn either susceptible or resistant to smut.

In the winter of 1925-26 some crosses of inbred lines of corn were tested in the greenhouse for smut resistance by the writer, using methods of inoculation slightly different from those of previous investigators (12), who found that lines of corn which were resistant to natural infection by smut in the field were also resistant in the greenhouse. Unexpectedly high percentages of smutted plants were obtained by the writer from a cross between naturally resistant lines of corn. In fact, the percentage of smutted plants resulting from the inoculations was about the same in the cross between the naturally resistant lines as in a cross between susceptible lines. This susceptibility of the cross between naturally resistant lines of corn led to the more detailed investigation of the problem reported in this paper.

## MATERIALS AND METHODS

Seed of selfed lines of the Garrick (C. I. 207),<sup>4</sup> Cuban (C. I. 218), and Boone County White (C. I. 240) varieties of corn and of crosses between them were used in both greenhouse and field experiments at the Arlington Experiment Farm, Rosslyn, Va. The seed was furnished by C. H. Kyle, agronomist in the Office of Cereal Crops and Diseases. Some of the lines had been selected for resistance and some for susceptibility to smut under field conditions at the Arlington farm.

Since a virulent culture of corn smut was desired, and since it was known that different cultures differ in pathogenicity (8, 12), the smut for the inoculations was obtained from different localities. In addition to the collections from the Arlington Experiment Farm, there were cultures from California, Pennsylvania, Tennessee, and

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<sup>2</sup> The writer is indebted to R. S. Talbott, who assisted in making the inoculations and in taking the smut data, and to A. M. Hurd-Karrer and B. O. Dodge, who made helpful suggestions in the preparation of the manuscript.

<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 89.

<sup>4</sup> Accession number, Office of Cereal Crops and Diseases.

Texas. The history of each smut collection, so far as known, is summarized in Table 1.

TABLE 1.—History of collections of *Ustilago zeae* used in experiments

Collection	Locality where collected	Year received	Cultured from—	Collection	Locality where collected	Year received	Cultured from—
No. 2.....	Rossllyn, Va.....	1922	Gall tissue.	No. 34.....	Rossllyn, Va.....	1925	Gall tissue.
No. 8.....	State College, Pa.	1923	Single spore.	No. 35.....	.....do.....	1925	Do.
No. 28.....	Tennessee.....	1923		No. 36.....	.....do.....	1925	Do.
No. 29.....	California.....	1923		No. 37.....	.....do.....	1925	Do.
No. 32.....	Rossllyn, Va.....	1922	Do.	No. 38.....	.....do.....	1925	Do.
No. 33.....	Dalhart, Tex.....	1924	Do.	No. 40-43 <sup>a</sup>	Rossllyn, Va. (greenhouse).	1926	Do.

<sup>a</sup> These collections were from galls from smutted plants of the naturally resistant cross, Garrick F54× Cuban F79, which had been inoculated with a conidial suspension from smut collection Nos. 8, 28, 32, 33, 35, and 38.

Conidial suspensions were used for all the inoculations, and these were obtained by growing pure cultures of the various collections in carrot decoction for 6 to 10 days. These conidial suspensions were mixed just before inoculation, except in one experiment in which they were used separately.

The plants were inoculated either by injecting the conidial suspensions into the young parts with a hypodermic syringe or by pouring the suspension into the tops of the plants before they had tasseled. In some cases both methods were used on the same plant.

## RESULTS

Experiments were conducted in the greenhouse in the winter of 1925-26 and in field plots in the summer of 1926 at the Arlington Experiment Farm.

### IN THE GREENHOUSE

In the first experiment plants of three F<sub>1</sub> crosses were inoculated. One was a cross between two naturally resistant parents, the second between a resistant and a susceptible parent, and the third between two susceptible parents. The parentage of these crosses is given in Table 2. Sixty seeds of each cross had been planted about every 8 inches in rows 9 inches apart on a greenhouse bench on December 4, 1925. The plantings were made in triplicate. The air temperature was kept close to 75°-80° F., although frequent fluctuations above and below these readings occurred.

The seedlings were inoculated when 18 days old, at which time they averaged less than a foot high. The age of the plants was reckoned from the date of planting throughout the investigation. Inoculation was made with a hypodermic syringe, using a mixture of conidial suspensions from several different smut collections (Nos. 32, 33, 34, 35, and 37). A method of inoculation described by Tisdale and Johnston (12) was used, the needle being inserted in the plant about 2 inches above the ground and the inoculum injected until it was forced out between the folded leaves at the top of the plant. In addition a quantity of the conidial suspension was poured into the top of each plant.

From five to seven days later chlorotic areas and incipient galls had appeared. The results of this experiment are given in Table 2.



TABLE 2.—*Reaction of 18-day-old corn seedlings in the greenhouse to infection by Ustilago zeae when a suspension of conidia was injected into them about 2 inches above the ground and an additional quantity poured into the top of each*

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Number of plants inoculated	Plants infected			
			With small galls or lesions on 1 or 2 leaves		With severe leaf or stalk galls	
			Number	Per cent	Number	Per cent
Garrick F54 × Cuban F79.....	R × R	158	34	21.5	3	1.9
Garrick F54 × Cuban F69.....	R × S	166	91	54.8	5	3.0
Garrick F90 × Cuban F69.....	S × S	163	54	33.1	1	.6

The data show that only 3 per cent or less of either the susceptible or the resistant crosses became severely smutted. Subsequent experiments, in which some of the smut collections were used individually for inoculations, produced high percentages of smutted plants in both resistant and susceptible lines. In addition to the severely smutted plants there were some which had either small galls or lesions on one or two leaves. The percentage of plants of the cross between resistant lines which showed these lesions or small galls was 21.5, of the cross between susceptible lines 33.1, and of the cross between susceptible and resistant lines 54.8 per cent, which indicates a difference in the resistance of the hybrids to the smut.

As the plants developed it was noted that the punctures made by the hypodermic needle 2 inches above the ground showed only in the leaves. Small galls often were present on a young leaf in the region of the needle puncture and on the tip of the next younger leaf which had not been punctured. It was obvious that the needle had not penetrated the stalk but only the tightly folded leaves. A longitudinal section through these plants showed that even at the age of 33 days (two weeks after inoculation) the apical bud was still near the level of the ground instead of 2 inches above, where the inoculations had been made. It was decided, therefore, to determine the effect of injecting the smut into the region of the apical bud. Accordingly, the severely smutted plants were removed, and the remaining plants, including those with small galls on the leaves, were reinoculated in the region of the apical bud on January 7, 1926. The needle was inserted in the plant about one-half inch from the ground, instead of 2 inches, as formerly, and the mixed conidial suspension was injected downward into the very young tissue. After 10 days, galls were appearing at the base, nodes, internodes, and on the leaves of many of these plants. The severely smutted plants were removed, and notes were made on the size, number, and location of galls on each plant.

The remaining plants, which were either smut free or had but very small galls on the leaves, were inoculated again on January 30, 1926, when about 8 weeks old, at which time the apical bud was several inches from the ground. Since the plants were larger and the internodes were elongating near the base, it was more difficult to estimate the location of the apical buds. All but four plants of each of two of the crosses became smutted as a result of this reinoculation of the tissue about the apical bud, and even these few unsmutted plants became smutted after being reinoculated on February 26, 1926.

The conidial suspensions for these three inoculations were mixtures from the same smut collections (Nos. 8, 28, 32, 33, 35, and 38). The results are shown graphically in Figure 1.

The data show that over 73 per cent of the plants of both the naturally resistant and the susceptible crosses, which were resistant to inoculation 2 inches above the ground, became smutted when the inoculum was injected about two weeks

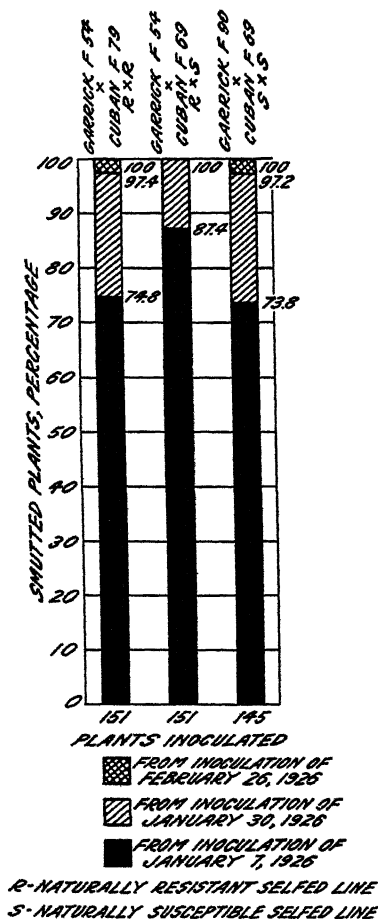


FIG. 1.—Percentages of smutted plants obtained when crosses of naturally resistant and susceptible lines of corn in the greenhouse were inoculated two or three different times by injecting conidial suspensions of *Ustilago zeae* into the region of the apical bud

later into the very young growing tissue about one-half inch above the ground. As a result of the reinoculation of the young tissue of the remaining smut-free plants, more than 97 per cent of the original number of plants became smutted. No plant of any cross remained free from smut after a third inoculation. As all of the plants of each cross became smutted, and as the smut for these three inoculations was from the same collections, it would appear that the plants of all crosses remaining smut free after the first and second inoculations were merely smut escaping.

Because of the unexpected susceptibility under artificial infection conditions of the naturally resistant cross, it was decided to test other lines of corn which are resistant to smut under natural conditions at the Arlington Experiment Farm. Accordingly, 40 seeds of each of five selfed lines and of a cross between two of these, all considered resistant to smut, were planted on a bench in the greenhouse on January 26, 1926. When the young seedlings were 1 month old and averaged less than a foot in height, they were inoculated about one-half inch above the ground in the young tissue about the apical bud. High percentages of plants of all the lines became smutted after this inoculation. Those plants which escaped infection were reinoculated on March 30.

The two plants remaining smut free after this second inoculation became diseased after a third inoculation on April 23.

The conidial suspensions for these three inoculations were from the same smut collections as those used in the previous experiment, except that collection No. 33 was not used in the second inoculation. The data obtained from this experiment are shown in Figure 2.

After the first inoculation, 100 per cent of the plants of two of these naturally resistant lines and over 74 per cent of the plants of the other four resistant lines became smutted. After the second inoculation the percentage of smutted plants in two of these four lines rose to 100, and the percentages of smutted plants of the other two lines became 96.8 and 97.1, respectively. All of the few remain-

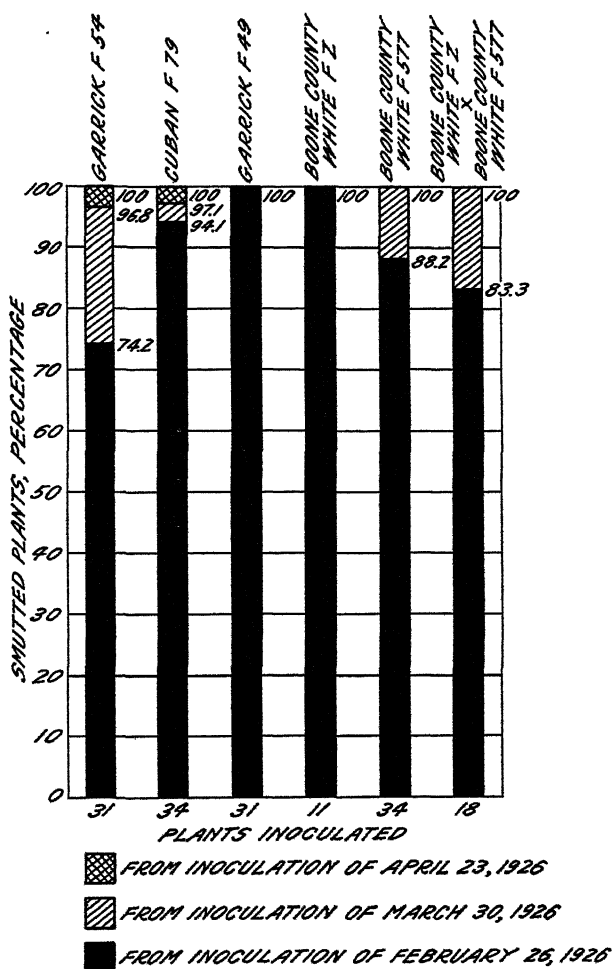


FIG. 2.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one, two, or three different times by injecting conidial suspensions of *Ustilago zeae* into the region of the apical bud before the period of tasseling

ing smut-free plants of these lines became smutted after the third inoculation.

In order to determine whether older plants of these same six lines of corn would become smutted after injections of conidia into the very young tissues, plants of these lines about 2½ months old were inoculated on April 23, 1926. Those plants which had not tasseled were inoculated in what was estimated to be the apical region of

the stalk. In plants which had tasseled the conidial suspension was injected through the leaf sheaths in attempts to inoculate the young bud tissues at the nodes. The percentages of smutted plants resulting from this inoculation ranged from 42.9 to 94.4. (Fig. 3.) A reinoculation of those plants which had escaped infection from the first inoculation was made on May 19, 1926. All plants with

the exception of a few dwarfed or stunted ones had tasseled, and the inoculum was injected into the ears and young growths from the nodes.

The conidial suspensions for these two inoculations were from the same smut collections used previously (Nos. 8, 28, 32, 33, 35, and 38). The results of the inoculations are shown in Figure 3.

The two inoculations resulted in 100 per cent of the plants of three lines becoming smutted, while the lowest final percentage of diseased plants in any line was 85. Evidently the age of the plant does not affect its susceptibility to smut except in so far as the development and growth of new tissue are concerned.

#### IN THE FIELD

It was thought possible that the very high percentages of smutted plants result-

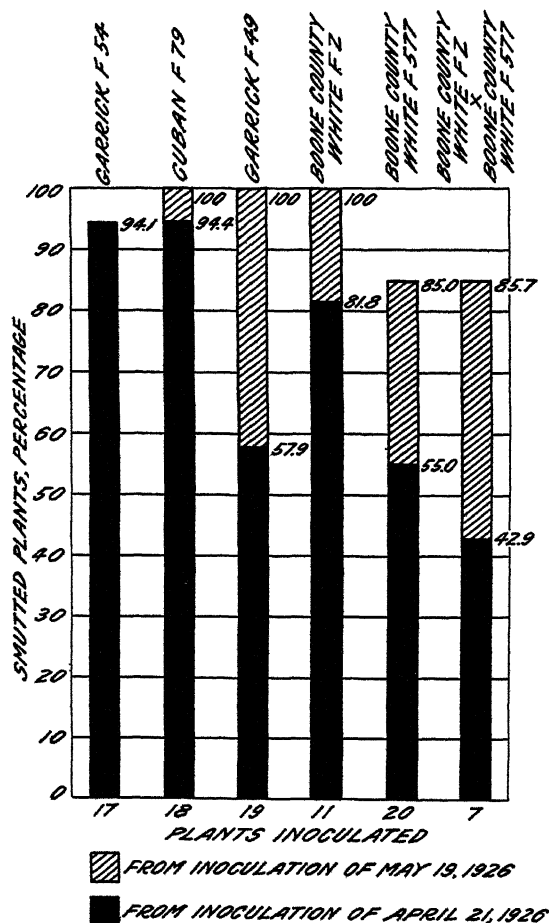


FIG. 3.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one or two different times by injecting conidial suspensions of *Ustilago zae* into the young tissues at about the period of tasseling

ing from the inoculation of naturally resistant lines of corn might be due to abnormal growth conditions in the greenhouse, and that in the field the plants might be resistant even if the inoculum were injected into the region of meristematic tissues. Accordingly, seeds of some of these same and other lines were planted in the field on May 20, 1926.

## INJECTION EXPERIMENTS

On July 2, when the plants were about 2 feet high, the plants of one row of each line were inoculated with a hypodermic needle as in the greenhouse experiments, using a mixture of smut collection Nos. 8, 33, 38, and 40 to 43. In order to place the inoculum in the region of the apical bud, the plants were inoculated about an inch above the ground. At the time of these inoculations smut galls had appeared on some of the plants as the result of natural infection. One row of plants of each strain was left uninoculated to serve as a check against naturally occurring infections.

Definite signs of infection as a result of the inoculations were noted in about six days. Later, the smutted plants in both the inoculated rows and the uninoculated control rows were removed. Plants with only small galls on one or two leaves were tagged but were not removed. They were counted as smut-free plants, as it was impossible to determine whether or not these slight infections resulted from the artificial inoculation. A second inoculation of the remaining smut-free plants was made on August 6, 1926, using a mixture of smut collection Nos. 29, 32, 33, 35, 36, 37, and 38. The plants at this time had not tasseled, except those of Garrick F54  $\times$  Cuban F79. Accordingly, the injections were made near the top of the plant in the youngest tissue. The location of this tissue had to be carefully estimated for each individual plant, as it naturally varied with the stage of development. As the plants of Garrick F54  $\times$  Cuban F79 had tasseled, the injections were made in the young ears and shoots only. The percentages of smutted plants are based on the total number of plants which became smutted up to and including each inoculation date. The smut percentages resulting from the two inoculations are shown in Figure 4, together with percentages of smutted plants appearing in the control rows in each of the same two periods.

The data in Figure 4 show that more than 70 per cent of the plants of every line except one were smutted as the result of the first inoculation. The one exception was Garrick F54, which had only 30.2 per cent smutted. This comparatively low percentage is interesting because the data in Figures 2 and 3 from previous experiments and the data in Tables 3 and 4 show relatively high percentages of smutted plants in this line.

The highest percentage of smutted plants in any of the uninoculated control rows of resistant lines for this period (July 2 to August 6) was 3.6, whereas in the corresponding uninoculated control rows of the susceptible lines, Garrick F90  $\times$  Cuban F69 and Cuban F69, smut percentages of 85.7 and 74.2 per cent, respectively, were obtained during the same period.

The final percentages of smutted plants resulting from the two inoculations were high in all the lines of corn tested. The percentages of smutted plants of the five naturally resistant lines totaled 100, 93.1, 91.5, 100, and 77.4 per cent, respectively. In the uninoculated control rows of these same lines the percentages of smutted plants were 5.3, 1.7, 18.0, 41.8, and 4.7 per cent, respectively. That there was no lack of opportunity for natural infection was shown by the very high percentages, 100 and 98.4, of smutted plants in the two uninoculated susceptible lines. Therefore, the high percentages of

smutted plants of these naturally resistant lines seem to have resulted from the method of inoculation, the essential element of which is evidently the injection of the smut conidia directly into the very young growing tissue.

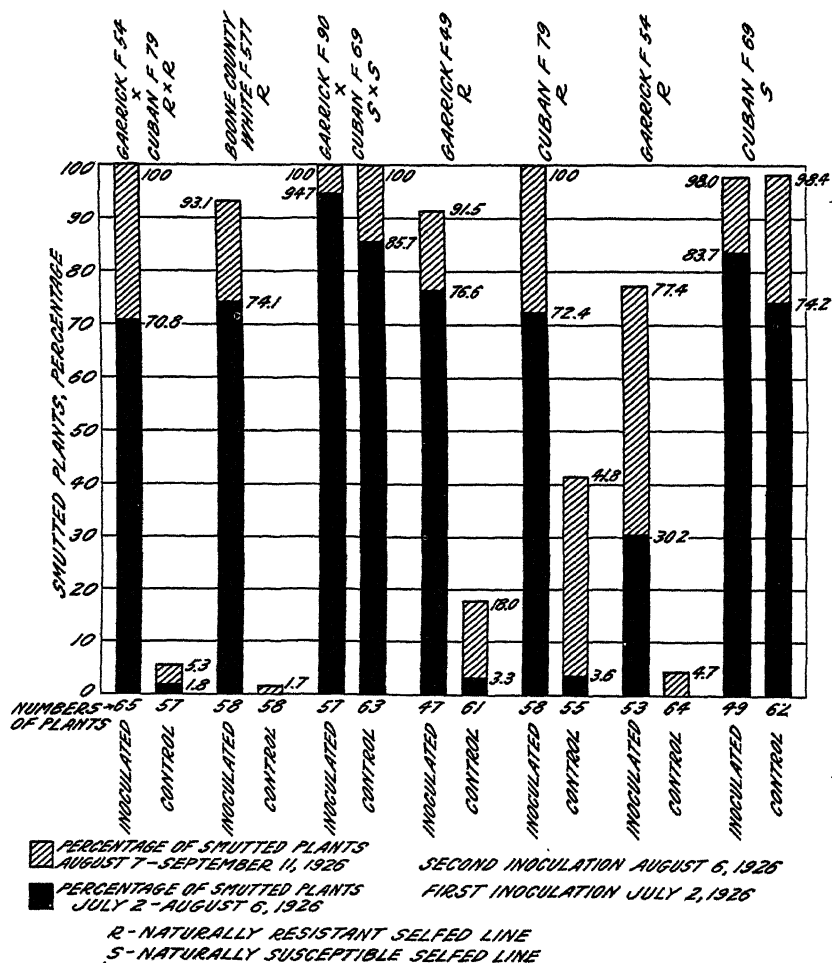


FIG. 4.—Percentages of smutted plants obtained in the field from naturally resistant and susceptible lines of corn when conidial suspensions of *Ustilago zeae* were injected two different times into very young tissue; together with percentages of smutted plants in the uninoculated controls

#### POURING AND INJECTION EXPERIMENTS

For the purpose of comparing the percentages of smut obtainable by the method of injecting the conidia directly into the meristematic tissue with those resulting from the commonly used method of pouring the conidial suspension into the top of the plant, young plants about 6 weeks old were inoculated, both methods being used. Inoculum from the same mixed conidial suspension, consisting of collection Nos. 29, 32, 33, 35, 36, 37, and 38, was used in each case. In about half of the plants the inoculum was injected into the stalk

about 1 inch above the ground, which was estimated to be the approximate location of the young growing point in plants of this age. In the others the plants were inoculated by pouring the conidial suspension into the top. At this date a few of the plants had large galls on the crown, nodes, and leaves, and these were discarded. The results of the inoculations are given in Table 3.

TABLE 3.—Comparative smut reaction in the field of plants of the same lines of corn when inoculated either by injecting a conidial suspension of *Ustilago zeae* into the apical bud tissue or by pouring the suspension into the top of the plant

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Plants inoculated by—									
		Injecting inoculum into young tissue					Pouring inoculum into top of plant				
		Total	Number smutted on—			Per-centage smutted	Total	Number smutted on—			Per-centage smutted
			Leaf only	Stalk only	Leaf and stalk			Leaf only	Stalk only	Leaf and stalk	
Garrick F54 × Cuban F79.....	R × R	34	0	1	33	100	32	0	3	0	9.4
Boone County White F577.....	R	27	2	2	20	88.8	21	0	0	0	0
Garrick F90 × Cuban F69.....	S × S	31	1	2	28	100	26	0	23	1	92.3
Garrick F49.....	R	27	1	3	16	74.1	25	0	1	0	4.0
Cuban F79.....	R	26	3	6	13	84.6	24	0	1	0	4.2
Garrick F54.....	R	30	4	1	12	56.7	30	0	0	0	0
Cuban F69.....	S	34	3	6	21	88.2	28	1	13	0	50.0

The lowest percentage of smutted plants in any of the five naturally resistant lines which were inoculated by injection of the spores into the young tissue was 56.7, while in one line all of the plants were smutted. One susceptible line had 88.2 and the other had 100 per cent of plants smutted.

The highest percentage of smutted plants of any naturally resistant line inoculated by pouring the inoculum in the top was 9.4, while two lines did not show any smutted plants. The corresponding percentages of smutted plants of the susceptible lines were 50 and 92.3.

Many of the susceptible plants inoculated by pouring spores into their tops were not smutted as a direct result of the artificial inoculation but as the result of natural infection, by which many of the buds at the lower nodes later became smutted. However, it was possible to determine that the plants inoculated by injection were smutted as a direct result of the inoculation, because the smut appeared only in the parts injected and within a short time. Since the same lines of corn were used and the smut in these inoculations was from the same mixed conidial suspension, the difference in the percentages of smutted plants obviously was due to the difference in the methods of inoculation.

#### INJECTIONS WITH LOCAL INOCULUM

The purpose of this investigation was to produce smut in naturally resistant lines of corn, yet in view of reports of the existence of physiologic forms of corn smut (8, 11), it seemed possible that the almost

complete susceptibility of the naturally resistant lines under artificial conditions might have resulted from the use of smut from localities other than the Arlington Experiment Farm. Accordingly, another series of inoculations was made with smut collected only at the Arlington farm, consisting of collection Nos. 2, 32, 34, 35, 37, and 38. As the plants available for these inoculations had tasseled, the inoculum was injected into the ears only. The ears were in different stages of development, some with the silk just beginning to dry and others in which the silk had not extruded from the husks. The row of each of the seven lines of corn was divided into seven sections. One section was inoculated with each of the six smut collections named above, and one was left as a control. Only plants showing smut in the ears were considered smutted as the result of the inoculations. The results, as well as the order of the six inoculations, are given in Table 4.

TABLE 4.—Number of smutted plants in naturally resistant or susceptible lines of corn in the field when conidial suspensions of six different collections of *Ustilago zae* from the Arlington Experiment Farm were injected into the ears

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Uninoculated control plants		Plants inoculated with—											
				Collection No. 38		Collection No. 2		Collection No. 37		Collection No. 35		Collection No. 34		Collection No. 32	
		Total	Smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted
Garrick F54 × Cuban F79.....	R × R	7	0	18	11	15	10	8	4	6	1	5	5	7	0
Boone County White F577.....	R	4	0	17	14	7	7	6	5	6	3	7	6	6	0
Garrick F90 × Cuban F69.....	S × S	2	0	3	3	3	3	1	1	2	1	2	2	4	0
Garrick F49.....	R	6	0	8	8	10	9	6	6	3	3	4	3	2	0
Cuban F79.....	R	5	0	4	4	9	9	3	3	3	1	7	6	5	0
Garrick F54.....	R	4	0	7	7	8	8	7	7	4	3	3	3	6	0
Cuban F69.....	S	2	0	3	3	1	1	1	1	1	1	3	3	5	0

The data in Table 4 show that the lowest percentages of smutted plants in any line except one were produced in the cross, Garrick F54 × Cuban F79, in which the ears were most mature at the time of inoculation. Three of the five naturally resistant lines inoculated with collection No. 2 had every plant smutted, one had 90, and the remaining one had 66.7 per cent smutted. Collection No. 2 was obtained from the Arlington Experiment Farm in 1922 and has been grown in culture since that year.

Differences in virulence of the different smut cultures were evidenced by the time of appearance of the galls, their size, and the extent of the invasion of the host tissue of the same line of corn. Collection No. 32 did not produce any smut even in the plants of the susceptible lines. The culture of smut for this inoculation grew normally in the carrot decoction. Collection No. 34 produced galls about two days earlier than the others. Collection No. 35 produced the smallest galls and the lowest percentage of smutted plants.



Although the probable importance of physiologic strains of corn smut from different localities in any corn-breeding program is recognized, it should be emphasized that the six different collections of smut from the rather localized area of the Arlington Experiment Farm showed differences in culture and in pathogenicity.

The susceptibility of these naturally resistant lines to artificial infection when inoculated with several collections of smut from the Arlington farm indicates that the high percentages of smut obtained throughout this investigation probably are not due to the fact that collections of smut from other localities were used in the inoculations, but rather to the fact that the inoculum was injected into very young tissue. Meristematic tissue of the lines tested is susceptible to smut even in plants of lines which are highly resistant to natural infection at the Arlington Experiment Farm.

#### DISCUSSION

In these experiments there apparently was little, if any, resistance of the very young host tissue to the smut. However, it is possible, of course, that there are physiological differences in the older tissues of the different lines of corn, which may determine their relative degree of resistance. Yet, in view of the fact that even in susceptible lines the galls are produced largely in the younger growing tissues, it is conceivable that resistance or susceptibility is largely a matter of relative accessibility of the susceptible parts to the invading organisms.

As has been shown, lines of corn developed at the Arlington Experiment Farm which were there resistant to natural smut infection in the field were extremely susceptible, under artificial inoculation, to several individual smut collections from the Arlington farm, as well as to mixed collections from different localities. These lines of corn as grown in the field at the Arlington farm in 1926 were resistant to natural infection by the smut occurring naturally there. (Fig. 4.) As stated previously, the naturally resistant lines used in these investigations were developed from only three varieties of corn. Consequently it is possible that the genetic factors involved in the selection of the various lines for resistance and susceptibility were limited.

If physiological differences in young tissue of different lines of corn are not determining factors in smut resistance, it is obvious that the injection method used in these experiments should not be employed for practical tests of the susceptibility of lines of corn to smut infection.

Recently Christensen and Stakman (1) have obtained differences in pathogenicity of physiologic forms of corn smut from different localities. Some of these forms were less virulent and some more virulent than the form from St. Paul, Minn., with which they were compared. These differences in pathogenicity perhaps would explain the susceptibility of the naturally resistant lines of corn developed at the Arlington Experiment Farm when inoculated with smuts from different localities, including the Arlington Experiment Farm. Yet even the existence of these forms of different pathogenicity from different localities does not explain the high percentages of smutted plants obtained in naturally resistant lines when several individual

smut collections from the Arlington Experiment Farm were used for the inoculations. (Table 4.)

It is possible that collections of smut from different localities would represent different physiologic forms. Yet even the different collections from the Arlington farm differed in infective power. Preliminary data concerning the pathogenicity of single-chlamydospore cultures of corn smut from the Arlington farm indicate that they differ even when obtained from the same smut gall.

It seems possible that some of the physiologic strains of *Ustilago zaeae* may be due to crossing within the species. Sartoris (9) reported the conjugation of the conidia of *U. zaeae* under certain growth conditions. It is not known whether these forms resulting from conjugation are pathogenic.

The significance of conjugation and hybridization in relation to physiologic forms of various species of fungi is apparent. Recent researches on smuts and other fungi indicate the advisability of using single chlamydospores, or even single conidia, of corn smut as the basis for determining physiologic forms of the organism. Kniep (7) has described and illustrated the conjugation between the germ tubes of sporidia of different species of *Ustilago* and the growth following these unions. Dickinson (2) recorded the occurrence of hyphal fusion within and across the two species of smut, *U. levis* and *U. hordei*. The pathogenicity of these new forms has not been demonstrated. Shear and Dodge (10) found that crossing the different species of the *Monilia sitophila* group by mating their haplonts resulted in the formation of what appeared to be hybrid perithecia with mature ascospores.

#### SUMMARY

Plants of selfed lines of corn and crosses between them which are resistant to natural infection by smut in the field at the Arlington Experiment Farm, Rosslyn, Va., were very susceptible when artificially inoculated in very young tissue. The apical and nodal bud tissues, immature ears, young leaves, and tassels were all highly susceptible when injected with conidial suspensions.

These lines were susceptible to several individual smut collections from the Arlington Experiment Farm as well as to mixed collections from there and from other localities. This fact indicates that the susceptibility of the naturally resistant lines need not be attributed to the use of physiologic forms from other localities.

#### LITERATURE CITED

- (1) CHRISTENSEN, J. J., and STAKMAN, E. C.  
1926. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN *USTILAGO ZAEAE*.  
*Phytopathology* 16: 979-999, illus.
- (2) DICKINSON, S.  
1927. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI.—HYPHAL FUSION. *Roy. Soc. [London] Proc. (B)* 101 (B 708): 126-136, illus.
- (3) GARBER, R. J., and QUISENBERRY, K. S.  
1925. BREEDING CORN FOR RESISTANCE TO SMUT (*USTILAGO ZAEAE*). *Jour. Amer. Soc. Agron.* 17: 132-140.
- (4) HAYES, H. K., STAKMAN, E. C., GRIFFEE, F., and CHRISTENSEN, J. J.  
1924. REACTIONS OF SELFED LINES OF MAIZE TO *USTILAGO ZAEAE*. *Phytopathology* 14: 268-280.

- 
- (5) IMMER, F. R., and CHRISTENSEN, J. J.  
1925. THE REACTION OF SELFED LINES AND CROSSES OF MAIZE TO *USTILAGO ZEAE*. *Phytopathology* 15: 699-707.
- (6) JONES, D. F.  
1918. SEGREGATION OF SUSCEPTIBILITY TO PARASITISM IN MAIZE. *Amer. Jour. Bot.* 5: 295-300.
- (7) KNIPE, H.  
1926. UEBER ARTKREUZUNGEN BEI BRANDPILZEN. *Ztschr. Pilzkunde* (n. F., Bd. 5) 10: 217-247, illus.
- (8) MELCHERS, L. E.  
1921. ECOLOGIC AND PHYSIOLOGIC NOTES ON CORN SMUT, *USTILAGO ZEAE*. (Abstract) *Phytopathology* 11: 32.
- (9) SARTORIS, G. B.  
1924. STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS. *Amer. Jour. Bot.* 11: 617-647, illus.
- (10) SHEAR, C. L., and DODGE, B. O.  
1927. LIFE HISTORIES AND HETEROTHALLISM OF THE RED BREAD-MOLD FUNGI OF THE *MONILIA SITOPHILA* GROUP. *Jour. Agr. Research* 34: 1019-1042, illus.
- (11) STAKMAN, E. C., and CHRISTENSEN, J. J.  
1926. PHYSIOLOGIC SPECIALIZATION OF *USTILAGO ZEAE* AND *PUCCINIA SORGHI*, AND THEIR RELATION TO CORN IMPROVEMENT. (Abstract) *Phytopathology* 16: 84.
- (12) TISDALE, W. H., and JOHNSTON, C. O.  
1926. A STUDY OF SMUT RESISTANCE IN CORN SEEDLINGS GROWN IN THE GREENHOUSE. *Jour. Agr. Research* 32: 649-668, illus.



# PRELIMINARY REPORT ON PARTIAL MEASUREMENTS OF FOREST PLANTATIONS<sup>1</sup>

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## INTRODUCTION

The measurement of forest plantations is a problem which has not as yet presented much difficulty in this country. However, as these plantations increase in area and the trees become larger, accurate measuring of the total volume will become more difficult. Adding to this the necessity of measuring such a stand periodically, it becomes essential that some method of accurately determining the total volume without actually measuring every tree be developed.

Forest plantations have certain characteristics that set them apart from native stands when the problem of volume mensuration is considered. One of the chief of these is the uniformity of plantations.

The trees are ordinarily planted in evenly spaced rows, and as a rule are regularly spaced in the rows. Plantations are usually even-aged and are composed of a limited number of species. These factors produce a degree of uniformity which is not attained in a native stand.

Growth studies in a plantation must be accurate if results are to be obtained which will be dependable in comparing the effect of spacing, pruning, etc., in plantings which are being given different treatment. In measuring a portion of a stand or "sampling,"<sup>2</sup> there is an error introduced when this portion is taken as representative of the whole. Returning to the same stand and measuring a different set of trees (although the same percentage of the whole) would probably give a different mean due to the variation in the individual trees. How, then, can the estimator be sure that the difference found between the two stands in question is not due to this error in sampling, and that the two stands are not actually equal in volume?

It goes without saying that measuring 50 per cent of the stand would give a mean which in most cases would be closer to the true mean than measuring 25 per cent or 10 per cent of the stand. And, likewise, 75 per cent would give better results than 50 per cent. But because of the difficulty of measuring a plantation—which is often greater than that of measuring a native stand—only as small a portion as is compatible with the desired degree of accuracy need be measured. If 50 per cent of the stand will give results which are practically as good as 100 per cent, then it is not necessary to measure more than 50 per cent, and if 25 per cent will suffice in a very uniform stand it is a waste of time to cover a larger percentage of the stand.

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<sup>1</sup> Received for publication Sept. 14, 1927; issued February, 1928.

<sup>2</sup> When measuring a portion of a stand it is essential that the sample be representative of the whole area and not merely chosen at random from any portion of the area. This is a basic principle in all methods requiring statistical treatment.

It is essential that the estimator determine the limit of accuracy desired before entering the stand. If an error of plus or minus 10 per cent of the total volume is allowable, a smaller percentage of the stand may be measured than if greater accuracy is desired.

A study was made in forest plantations in Ohio to determine the percentage of a stand which it is necessary to measure in order to obtain sufficiently accurate results. This study was made chiefly in white pine plantations of various spacings, but also included plantations of red pine, Scotch pine, white ash, and red oak. The stands were located at Oberlin in the northern part of the State, at Wooster in the central, and at Athens in the southern part.

### EXPERIMENTAL METHODS

The method of study was as follows: Each tree in the stand was measured for height and diameter, and a separate tally kept for each row. The averages of height and diameter were found for each stand, and the averages were then computed for various percentages of each stand by taking a certain number of rows and computing the averages for the trees in these rows. For instance, the average of 50 per cent of the stand was found by using the trees in alternate rows, and of 25 per cent by using every fourth row, etc. This method was purely mechanical in application, thus eliminating the personal factor of judgment.

The standard deviations of the two variants, height and diameter, were computed for the different percentages of the stand, and from the standard deviation the probable error was determined for each percentage. A hypothetical case (Table 1) illustrates the method used:

TABLE 1.—Standard deviations and probable errors of heights and diameters

Tree No.	Height	Deviation from assumed means	Deviation squared	Diameter	Deviation from assumed means	Deviation squared
	<i>Feet</i>			<i>Inches</i>		
1.....	21.5	3.5	12.25	2.3	0.2	0.04
2.....	26.0	1.0	1.00	2.7	.2	.04
3.....	23.0	2.0	4.00	2.4	.1	.01
4.....	22.5	2.5	6.25	2.3	.2	.04
5.....	29.0	4.0	16.00	3.0	.5	.25
6.....	27.0	2.0	4.00	2.7	.2	.04
7.....	26.5	1.5	2.25	2.5	.0	.00
8.....	30.0	5.0	25.00	3.1	.6	.36
9.....	23.5	1.5	2.25	2.2	.3	.09
10.....	24.0	1.0	1.00	2.5	.0	.00
11.....	22.0	3.0	9.00	2.2	.3	.09
Total.....	275.0		83.00	27.90		.96
Average.....	25.0			2.54		

Assumed mean height=25.0 feet.

Assumed mean of diameter=2.5 inches.

$$\text{Standard deviation}^3 = \sqrt{\frac{\Sigma d^2 - na^2}{n}} \quad \text{when—}$$

$\Sigma d^2$  = the sum of the squares of the deviations (from the assumed mean).

$a$  = difference between the assumed mean and the computed mean.

$n$  = number of trees.

$$\text{Standard deviation of height} = \sqrt{\frac{83 - 11 \times 0}{11}} = \sqrt{6.55} = 2.55 \text{ feet.}$$

$$\text{Standard deviation of diameter} = \sqrt{\frac{.96 - 11 \times .04^2}{11}} = \sqrt{.08} = .28 \text{ inch.}$$

$$\text{Probable error (P. E.)} = .6745 \frac{\text{Standard deviation.}^4}{\sqrt{n}}$$

$$\text{Probable error of height} = .6745 \frac{2.55}{\sqrt{11}} = .52 \text{ foot.}$$

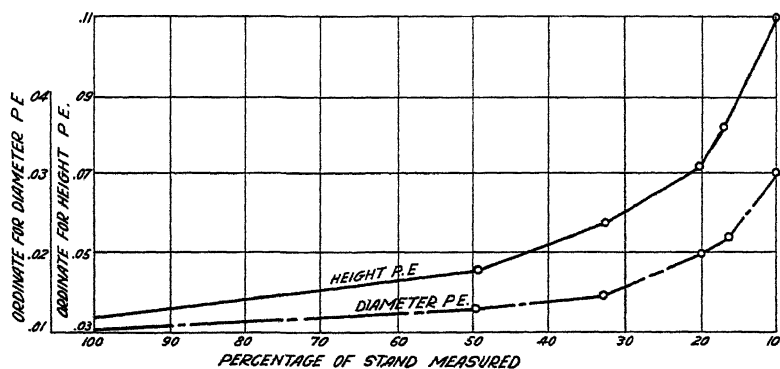


FIG. 1.—Curves for red pine plot, spacing of trees 3 × 3 feet, showing the probable error of the mean of the heights and diameters

$$\text{Probable error of diameter} = .6745 \frac{.2}{\sqrt{11}} = .041 \text{ inch.}$$

The probable error indicates the degree of accuracy attained. To illustrate: The chance of a deviation from the computed mean being greater than the probable error is equal to the chance that it will be less, and the chances are about 22 to 1 that the deviation will not exceed three times the probable error. (Differences between two means which are further apart than three times the probable error<sup>5</sup> have been accepted by the majority of biometricians as being significant and not due to errors in sampling.)

<sup>3</sup> PHILLIPS, F. M. SHORT METHOD OF OBTAINING A PEARSON COEFFICIENT OF CORRELATION, AND OTHER SHORT STATISTICAL PROCESSES. U. S. Mo. Weather Rev. 50: 135-136. 1922.

<sup>4</sup> YULE, G. U. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 7, rev., 415 p., illus. London. 1924.

<sup>5</sup> In this case the probable error used is the probable error of the difference, or:

$$(\text{Probable error})_{a-b} = \sqrt{(\text{P.E.})_a^2 + (\text{P.E.})_b^2}$$

## EXPERIMENTAL RESULTS

The results of the study in the stands are summarized in Tables 2 to 5. Space does not permit the printing of the curves for each plot, but those for a fairly representative plot are shown to demonstrate the trend of the probable error as diminishing percentages of the stand are measured.

TABLE 2.—*Probable error of mean of diameter (in inches)*

Plot	Spacing of trees	Percentage of stand measured								
		100	80	66	50	44	33	25	20	10
		<i>Feet</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>	<i>Inch</i>
White pine.....	4 by 4	0.013			0.017		0.020	0.031		
Do.....	3 by 6	.030			.040		.057	.065		
Do.....	6 by 6	.078		0.062		0.110		.160		
Do.....	6 by 12	.052	0.060		.075		.100			
Do.....	3 by 3	.056		.070	.075		.120	.120		
Do.....	3 by 3	.020			.028		.032	.037		
Scotch pine.....	3 by 6	.030			.037		.047		0.062	
Do.....	3 by 3	.028		.032	.049		.051	.052		
Red pine.....	3 by 3	.010			.013		.015		.020	0.030
Red oak.....	6 by 6	.034	.042		.042		.060			.065
White ash.....	4 by 4	.052				.076	.092		.108	

TABLE 3.—*Probable error of mean of height (in feet)*

Plot	Spacing of trees	Percentage of stand measured								
		100	80	66	50	44	33	25	20	10
		<i>Feet</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>	<i>Foot</i>
White pine.....	4 by 4	0.067			0.062		0.120	0.17		
Do.....	3 by 6	.140			.190		.250	.28		
Do.....	6 by 6	.180		0.26		0.23		.30		
Do.....	6 by 12	.140	0.16		.200		.340			
Do.....	3 by 3	.150		.17	.220		.250	.31		
Do.....	3 by 3	.080			.130		.170	.19		
Scotch pine.....	3 by 6	.090			.120		.160		0.200	
Do.....	3 by 3	.075		.09	.110		.140	.16		
Red pine.....	3 by 3	.034			.046		.058		.072	0.11
Red oak.....	6 by 6	.170	.19		.240			.35		.48
White ash.....	4 by 4	.380				.45	.480		.610	

The limit of accuracy to be desired in forest plantations will vary somewhat, but, for the most part, variation in results should not greatly exceed the degree of accuracy in the field work, viz,  $\pm 0.1$  inch in the mean of diameter and  $\pm 0.5$  foot in the mean of height. With these figures in mind the above results may be analyzed and the percentage of each stand which it would have been necessary to measure to obtain this degree of accuracy calculated. Since the true mean will usually (22 times out of 23) be found within the limits of plus or minus three times the probable error, the desired accuracy restricts the probable error to one-third the limits decided upon, or to approximately 0.2 foot in height and 0.04 inch in diameter.

Of the 11 stands studied, 4 must be measured entirely in order to obtain the desired accuracy in diameter, 4 need only a 50 per cent estimate, 2 a 25 per cent estimate, and 1 a 10 per cent estimate. In order to obtain the desired accuracy in height 2 must have a 100 per cent estimate, 1 an 80 per cent, 3 a 50 per cent, 3 a 25 per cent, 1 a 20 per cent, and 1 a 10 per cent estimate.



TABLE 4.—Percentage of each stand which must be measured in order to obtain the desired accuracy in diameter and height

Plot	Spacing of trees	Location	Total number of trees in each stand	Diameter Per cent	Height Per cent
White pine.....	4 by 4	Athens.....	612	25	25
Do.....	3 by 6	Wooster.....	404	50	50
Do.....	6 by 6	Oberlin.....	140	100	100
Do.....	6 by 12	do.....	360	100	50
Do.....	3 by 3	do.....	230	100	50
Do.....	3 by 6	Wooster.....	725	25	25
Scotch pine.....	3 by 3	do.....	400	50	25
Do.....	3 by 3	do.....	465	50	25
Red pine.....	3 by 3	do.....	1,155	10	10
Red oak.....	6 by 6	do.....	270	50	80
White ash.....	4 by 4	do.....	400	100	100

From these facts it is apparent that in small, nonuniform stands such as the Oberlin and the white ash stands, less than a 100 per cent estimate will not give the desired accuracy in diameter. In the majority of small but uniform stands, 50 per cent will give accurate results, and in uniform stands of over 500 trees a third or a quarter of the stand will give good results.

The limit of accuracy in height allows for a smaller percentage of the stand to be estimated in some cases than does the limit of accuracy in diameter; in one instance only was it found that more of the

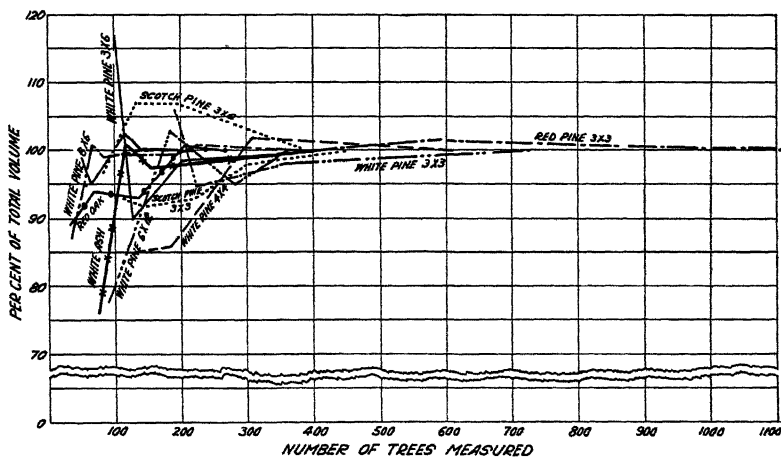


FIG. 2.—Composite curve showing the relation between number of trees measured and accuracy in volume

stand should be measured for height than for diameter. These figures, while they are not final and do not justify definite conclusions on this matter, suggest the possibility that fewer trees need to be measured for height than for diameter in order to obtain the desired accuracy.

The ultimate aim of most timber estimates is to determine the volume of the tree or stand. This volume is affected by two variables, viz, height and diameter, and these two variables do not always have a constant relationship. It is possible, therefore, that any variation from the mean of one variable may compensate for the variation in the other, and thus tend to maintain an estimated volume which is

nearer the true volume. If this is the case, a smaller percentage of the stand might be measured than that allowed by the probable error of either height or diameter.

Following out this theory, the volumes of the stands studied were computed and also the volumes for the various percentages of each. These were then plotted on a common graph, using the number of trees measured as the abscissa and the percentage of the total true volume as the ordinate. This produced a series of curves (fig. 2), each independent of the others, but all based on the same ordinates.

A glance at these curves brings out the fact that between 200 and 400 trees there is a sharp trend toward a common line. As the number of trees measured increases, the volumes found approach more and more closely the true or 100 per cent volume. Thus, measuring 150 trees in the stands in question gives a volume that is within 9 per cent of the true volume in all cases, 250 trees give a result that is within 5 per cent, and 350 trees give a result within 2 per cent of the true volume.

Allowing an error of  $\pm 5$  per cent of the true volume, measuring approximately 250 trees in any of the stands in question would give satisfactory results. In uniform stands, which are about the same size as those studied in this problem, the measuring of a representative percentage of the stand which will give about 250 trees should give results which will not vary from the true volume by more than 5 per cent.

TABLE 5.—Number of trees in each plot, percentage estimate needed, and number of trees this estimate will include

Plot	Spacing of trees	Total number of trees	Percentage estimate needed	Number of trees to be measured
	<i>Feet</i>			
White pine.....	4 by 4	612	25	153
Do.....	3 by 6	404	50	202
Do.....	6 by 6	140	100	140
Do.....	6 by 12	360	100	360
Do.....	3 by 3	230	100	230
Do.....	3 by 3	725	25	181
Scotch pine.....	3 by 6	400	50	200
Do.....	3 by 3	465	50	233
Red oak.....	6 by 6	270	80	216
White ash.....	4 by 4	400	100	400
Red pine.....	3 by 3	1,155	10	115

Applying this rule, namely, the need of measuring approximately 250 trees in a stand, to Table 5, it is found that in only two stands did the limit of accuracy as determined by the probable error of both diameter and height call for an estimate of more than 250 trees in a stand. This rule of thumb apparently is compatible with the accuracy attained by keeping the probable error of height within  $\pm 0.2$  foot and the probable error of diameter within  $\pm 0.04$  inch. In the stands studied, therefore, measuring a percentage of the stand which will include at least 250 trees will give results which are within the limits of accuracy as determined by both volume and probable errors.

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NO. 2

## THE DIRECT EFFECT OF POLLEN ON THE FRUIT OF THE DATE PALM<sup>1</sup>

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### INTRODUCTION

The culture of the date palm is unique in many respects, as compared with that of the more familiar tree crops of the Temperate Zones. Not the least picturesque of the operations which characterize the industry is that of artificial pollination, necessary because of the dioecious nature of *Phoenix dactylifera* and the impracticability under commercial conditions of resorting to the natural method of wind pollination. The practice is as old as date culture itself. Obviously any direct influence which pollen may have upon the fruit is of immense practical importance to the date grower, for if available it is just as easy to use one pollen as another.

The variability of fruit-bearing palms grown from seed, the original source of all cultivated varieties, has been recognized from the beginning. It has been emphasized over and over again by the experiences of growers who have attempted to establish commercial gardens in this way and who have seldom found more than 1 or 2 per cent of such palms worthy of further propagation. Yet very little care has been used in the selection of palms for pollen. Although a few individual male offshoots came in with some of the earlier importations, at present no varieties have been established in this country. Seedling males have been used more or less indiscriminately and a good setting of fruit has generally been regarded as all that could be expected. Of course, obvious physiological differences among individual male palms have compelled attention. Because of wide variations in the time of blooming, size and number of inflorescences, quantity of pollen produced, etc., many are of little or no value.

That pollen might influence directly the fruit of the date palm has not been generally believed either by date growers or by botanists. Isolated statements without the confirmation of experimental data attract little attention. Popenoe<sup>2</sup> cites Schweinfurth<sup>3</sup> as having "declared that the characteristics of the male had an influence on the fruit which resulted." From the original it appears that Schweinfurth commented only on the variability in the size of the seed, but mentioned no other effect on the fruit. Popenoe<sup>2</sup> reports informally work done by Bruce Drummond when he was superintendent of the

<sup>1</sup> Received for publication Aug. 29, 1927; issued March, 1928.

<sup>2</sup> POPENOE, P. B. DATE GROWING IN THE OLD WORLD AND THE NEW. p. 108-109. Altadena, Calif. 1913.

<sup>3</sup> SCHWEINFURTH, G. UEBER DIE KULTUR DER DATTELPALME. Gartenflora 50: 513. 1901.

United States experiment date garden at Indio, Calif. Pollen of *Phoenix canariensis* is said to have influenced the quality of the fruit of the Rhars variety, producing a better date than was obtained on this same variety with pollen of *P. dactylifera*. In addition, on the authority of Drummond, it is reported by Popenoe that "a difference of as much as one-third in the size and of 20 days in the time of ripening seems to have been due to a change in the male used for pollinating." Drummond in his official reports to the Washington office (unpublished manuscripts) attributed marked effects on the time of ripening exerted on dates by pollen of certain male palms where both parents were cultivated varieties of *P. dactylifera*. However, in the absence of detailed records of experiments safeguarded against any possible source of error, these supposed effects remained more or less dubious, and other problems of more immediate concern in the establishment of the date industry demanded attention.

In order to throw some light on the question, a series of experiments was begun at the United States Experiment Date Garden, Indio, Calif., in the spring of 1925 and repeated on a more extensive scale in 1926.

#### POLLENS TESTED

As the basis of these experiments two *dactylifera* males were selected which seemed most likely to differ in their influence upon the fruit.

Fard No. 4 (fig. 1, A, and fig. 2, B) was grown from seed of imported fruit by Fred N. Johnson at Indio, Calif., and transplanted to the United States Experiment Date Garden in 1910. A description of this palm follows:

Height, 17 feet (measured to the tip of the bud as noted from the last fiber visible at the base of the youngest leaves); length of leaves, 10 to 12 feet; diameter of trunk, 26 inches (measured 3 feet above the ground and including fiber and closely pruned stubs of leaf petioles); has one offshoot remaining; in 1925 produced 14 spathes from February 6 to April 11, with two later ones the latter part of June. Though at present it is not grown in this country, the small to medium sized brownish black fruit of the Fard variety is well known to connoisseurs of dates, as it has long been imported in considerable quantities from eastern Arabia.

Mosque (fig. 1, B, and fig. 2, A) was grown from seed obtained by S. C. Mason near Kena, below Luxor, Egypt, in 1913 and planted at Indio, Calif., in 1914. A description of this palm follows:

Height, 19 feet; length of leaves, 16 to 20 feet; diameter of trunk, 31 inches; has one offshoot remaining; in 1925 produced 22 spathes from February 6 to March 24. This is a very vigorous male producing an abundance of pollen in spathes nearly twice as large as those of any other male at the United States Experiment Date Garden. Curiously enough, in view of the results obtained with pollen from this palm, Mason reports that the parent palm produced fruit which, though of excellent quality, was of only medium size.

In 1925 pollens from three other *dactylifera* palms (figs. 1 and 3) in addition to the two described were also tested, along with pollen from a *Phoenix canariensis* (Canariensis No. 1). The well-known Canary Island palm is very common in ornamental plantings in southern California. The pollen was obtained from a palm along a driveway in a neighboring community. In 1926, in addition to Mosque and Fard No. 4, pollens from 19 *dactylifera* males were tested along with pollens from two individuals of *P. canariensis*—Canariensis No. 2, a palm growing on the grounds of the Imperial County courthouse at El Centro, Calif., and Canariensis No. 3, a palm growing



FIG. 1.—Male palms whose pollens were tested: A, Fard No. 4; B, left, Deglet Noor R-6; center, Mosque

along the highway near Brawley, Calif. All of the male palms tested are of seedling origin.

Since there are likely to be differences between fruit produced on different palms of the same variety and even on the same palm to some extent between different bunches, especially as between early and late inflorescences, comparative pollinations are best made within a short range of time and on the same palm. With this in view, eight experiments were made in 1925. Seven of these were on three Deglet Noor palms, Nos. 2-8-1, 2-9-1, and 2-5-7, 15 to 16 years of age, growing at the United States Experiment Date Garden and in

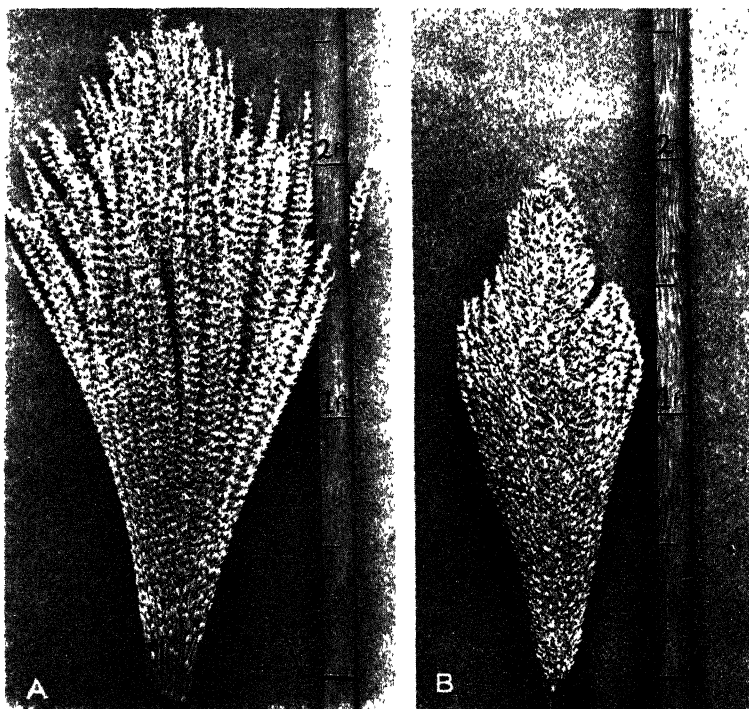


FIG. 2.—Staminate inflorescences from palms tested (the protecting sheath or spathe has been removed): A, Mosque; B, Fard No. 4

full commercial bearing. The other experiment was on Deglet Noor seedling No. 6, approximately the same age as the other three. In 1926, 19 experiments were made—10 on the 3 Deglet Noor palms mentioned above, 5 on 4 other Deglet Noor palms; 1 on Deglet Noor seedling No. 6, and 1 each on palms of the Rhars, Khadrawy, and Maktum varieties. Mosque and Fard No. 4 pollens were included in all of the experiments in 1925 and in 15 experiments in 1926; Canariensis No. 1 in 6 experiments in 1925; Canariensis No. 2 in 5 experiments and Canariensis No. 3 in 2 experiments in 1926. Along with the foregoing the other *dactylifera* pollens, comprising a total of 20 for the 2 seasons, were each tested in from 1 to 4 experiments.

In addition to those enumerated at the Indio station, nine experiments with the Mosque and Fard No. 4 pollens were made in the



FIG. 3.—Male palms whose pollens were tested: A, Deglet Noor N-12; B, Fard FNJ-N

Salt River Valley, Ariz., in 1926—seven on two Deglet Noor palms in full bearing at the Tempe Date Garden and one on Deglet Noor and one on Iteema at the garden of the Arizona Orchards Co. The Mosque and Fard No. 4 pollens were compared directly in 30 experiments.

#### CARE OF POLLEN

Owing to the fine powdery nature of date pollen, special precautions are necessary in handling it. The male palms used in these tests were visited every morning and mature spathes cut as soon as they showed signs of splitting or opening. During the first season, to minimize the danger of contamination from pollen blown about in the air or transported by bees from other male palms, no spathes were allowed to mature on any male palms except those to be tested, the others being cut from the palm before opening and while still immature.

This was not done during the second season. Instead, along with improved technic in pollination and greater precautions in the field, the better method was adopted of bagging such spathes as were desired prior to opening, mostly with glassine paper bags through which the opening of the spathe could be easily observed. Each pollen was laid out to dry in shallow trays and kept locked in a separate room reached from a different entrance. To avoid storage complications most of the pollens used in only one or two experiments were gathered, prepared, and used immediately. After handling one pollen, whether cutting the spathe for storage or using it for pollination, the operator was very careful to change clothes and to wash all exposed portions of the body. Precautions were also taken with all implements used.

#### TECHNIC OF POLLINATION

It is difficult to bag satisfactorily an entire cluster of female flowers. When the spathe first begins to open, the basal flowers on the strands within are usually still far down in the axil of the leaf. Fortunately, the Deglet Noor produces spathes longer and narrower than most other varieties. To cover these spathes, long narrow paper bags were made of heavy brown wrapping paper, two thicknesses each, sealed separately—essentially two bags, one within the other. A close watch was kept on the growing spathes from day to day, and each flower cluster was pollinated as soon as the spathe showed the slightest tendency to crack or open. In several of the 1926 experiments the spathes were broken apart and pollination accomplished a day or two, as nearly as could be estimated, before they would have opened normally. The results of such pollinations were entirely satisfactory. In most of the other experiments at Indio in 1926 the spathes were sponged with alcohol and several days before opening covered with glassine bags, which were removed at the time of pollination.

In pollinating, a band of cotton was first tied tightly around the base of the spathe as far down in the axil of the leaf as possible. The sides of the spathe were then pulled apart and pollen applied with a tuft of cotton about the size of a walnut, three or four being placed at different elevations between the strands. After the sharp edges



were trimmed the spathe itself was left to give rigidity to the bag, which was placed over all and tied firmly to the band of cotton at the base. As a further precaution when the basal flowers were very far down in the axil of the leaf, a second band of cotton was tied around the outside and as far down at the base as it was possible to push it. The bags were examined from time to time and during the first two weeks were pushed farther down into the axil of the leaf whenever necessary to prevent exposure of the inclosed inflorescence because of the elongation of the fruit stalk.

#### APPLYING POLLENS FROM SEVERAL SOURCES TO THE SAME INFLORESCENCE

Under any conditions there are apt to be slight variations between the fruit of different bunches due to variations in exposure, time of blooming, etc. Hence it is very desirable for comparison to apply the different pollens on the same inflorescence. Under such conditions greater care must be used to prevent contamination, but this is partially offset by the greater efficiency of bagging, for it is possible to put cotton all around each individual strand when only a few are used and there is no danger of subsequent exposure because of the growth of the fruit stalk.

Several pollens were applied to different strands on the same inflorescence in three of the experiments on Deglet Noor in 1925 (Nos. 4, 5, and 6). In 1926 this means of comparison was used in all of the experiments except four on Deglet Noor (Nos. 1 to 4, inclusive) at the Indio station and two on Deglet Noor (Nos. 1 and 6) at the Tempe Date Garden.

In 1925 sets of three strands each were chosen and inclosed in long narrow paper bags before any pollen was applied. As additional protection, the entire inflorescence was then inclosed in a canvas hood. In pollinating, one bag was removed from under the hood and taken off the strands; pollen was applied on all the flowers with a tuft of cotton, using a superabundance of pollen; then the strands were rebagged and left outside the canvas hood until the next pollination, or about two hours, some such interval being considered desirable to permit the wind to remove pollen which might remain in suspension in the air about the inflorescence.

#### A NEW METHOD FOR APPLYING ANEMOPHILOUS POLLENS

In 1926 a method was worked out for applying several pollens to different strands on the same inflorescence which greatly facilitates field work and insures a minimum of contamination. This consists in sealing the pollens in small paper packets, about  $2\frac{1}{2}$  by 5 inches, and gluing these small packets within the larger bags, about 3 by 24 inches, at the upper or sealed end. After the large bag has been placed over the strands to be pollinated and the lower or open end plugged with cotton and tied, the pollen is released by pulling a copper wire attached to a small tuft of cotton inside the sealed packet that contains the pollen. This breaks the pollen packet and releases pollen so it falls on the female flowers. This is not done until all of the bags are in position (fig. 4), and is accomplished by holding the upper end of the bag with one hand and pulling the lower end of the copper wire with the other. The small packets used were made of heavy glassine paper, two thicknesses each sealed separately, and

before being placed within the pollination bags they were washed in a strong solution of bichloride of mercury. The 3 by 24 inch bags were similarly made of two thicknesses of glassine paper, each sealed separately, and it was possible to observe the action of the wire plunger in effecting pollination.

This method eliminates the necessity for direct contact with pollen in the field, and the pistillate flowers are exposed only during the few moments while the bags are being placed in position. As was

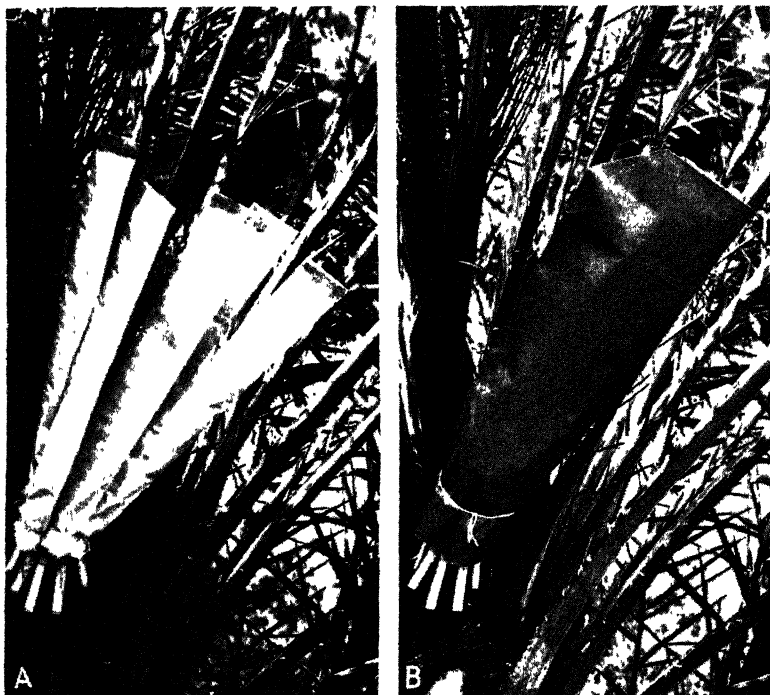


FIG. 4.—Type of bags used in experiments with different pollens on the same inflorescence. Each bag in A incloses a set of strands. Emerging from the bags at the base are the copper wires used to release pollen sealed in small packets at the upper end within. B shows a larger bag placed over the others for additional protection

done in these experiments, it is advisable to protect all the bags on a single inflorescence with a larger bag or hood.

#### EFFICIENCY OF TECHNIC

In the 1925 experiments two entire bunches were bagged without the application of any pollen. In one of these unpollinated treatments 1,755 dates developed, of which 14 (0.79 per cent) contained seed; in the other 1,266 dates developed, of which 32 (2.5 per cent) contained seed. In three similar unpollinated treatments in 1926 seeds were contained as follows: 5 out of 2,106, 3 out of 1,104 and 7 out of 588—almost negligible except the last, 1.2 per cent. This may be regarded as an approximate indication of the efficiency of this type of bagging; but it should also be stated that since both of the clusters in 1925 and the last mentioned in 1926 were not bagged

until the morning when the first indication of opening was observed, it is probable that in these three cases some of the pollen reached the flower clusters before they were bagged. This was indicated by the fact that the dates which contained seed were on that portion of the cluster which was first exposed by the opening of the spathe.

Each pistillate date flower contains three ovules. When pollinated, only one of these normally develops to maturity; the other two usually dry up and slough off. In most varieties if the unpollinated dates develop at all there is a slight but more or less equal development of all three ovules, sometimes crowding on the strand, resulting in a thick mass of miniature seedless dates. In the Deglet Noor variety the development of a large percentage of single dates on unpollinated inflorescences seems to be characteristic, although unpollinated inflorescences are not likely to develop as many dates as pollinated ones.

In each of the experiments at the Indio station in which several pollens were applied to the same inflorescence, an unpollinated treatment was included, to determine the degree of exposure to foreign pollen. In these, with a few exceptions, not many unpollinated dates developed, so that the proportion of dates with seed to those without would not be even an approximate indication of the efficiency of technic. However, out of 18 experiments at Indio only 4 of the unpollinated treatments developed any seed at all, and except for 4 in No. 13 in 1926 there was only 1 in each of these—No. 4 in 1925 and Nos. 14 and 17 in 1926. It does not appear, therefore, that sufficient foreign pollen could have reached the stigmas to have seriously impaired the reliability of the series of experiments. In the few cases mentioned it is very likely that air currents carried the pollen to the pistillate flowers during the few moments of exposure before they were bagged.

#### SEEDS PRODUCED BY CANARIENSIS POLLEN

Another indication of the care taken in handling the various pollens was afforded by a study of the seed resulting from the *canariensis* pollinations. These were found not only to average smaller than the seed resulting from any other pollination but also to have a characteristic tapering toward the basal end. (Fig. 5, A.) In the *canariensis* pollinations in 1925, of the three experiments containing this pollen on the same inflorescence with others, two had no off-type seed, but the third had 3 out of 30 which appeared to be the result of foreign pollen, or a possible contamination of 10 per cent. In the other three *canariensis* pollinations, each representing an entire bunch, the first had 3 dates which probably received foreign pollen; the second, 3; and the third only 1 out of 100 dates examined in each experiment. In 1926 out of 30 dates taken at random from each of the 7 experiments in which *canariensis* pollen was used, none of them were found to contain off-type seed. In one of these experiments (No. 3) 2 out of the 30 seeds examined were rough coated, but while slightly larger than the others there was not sufficient difference in size or shape to make it certain that other pollen had been introduced.

On the other hand, as another possible indication of the care taken in handling the various pollens, it should be noted that in all of the other experiments no seed resulted from the *dactylifera* pollinations which resembled those produced by the *canariensis*.

However, in judging the relative purity of pollination by the appearance of the seed, while the foregoing conclusions seem probable, the assumption is not justified that because a characteristic size or shape occasionally fails to appear some other pollen is necessarily responsible. Possibly the only absolute test of the purity of date pollinations would be the palms which the seed in question produced. To

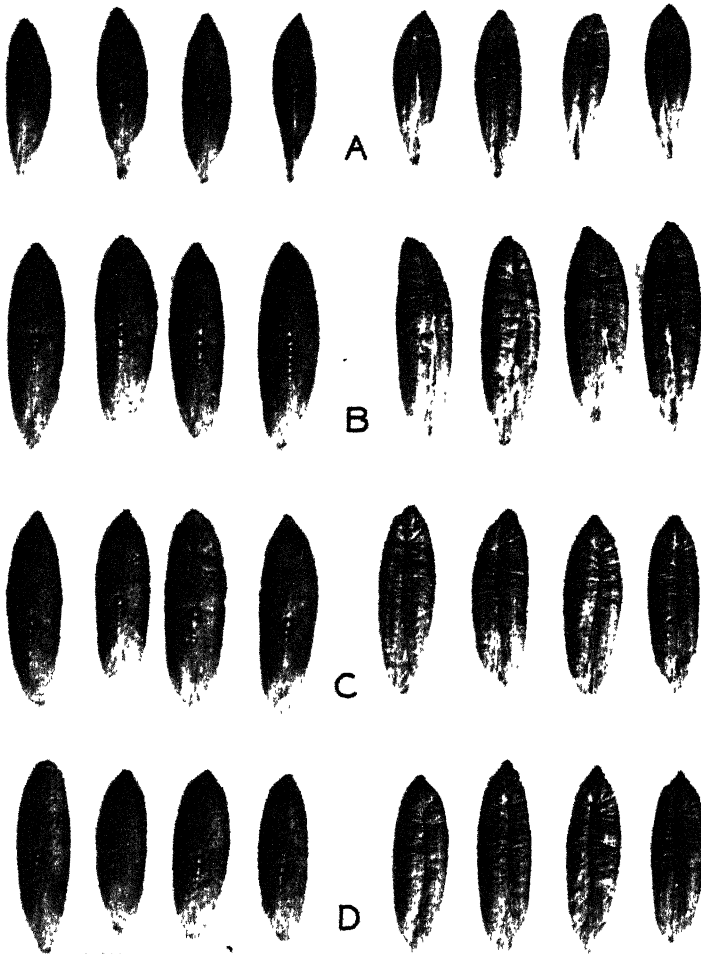


FIG. 5.—Typical Deglet Noor seeds produced on the same cluster by different pollens in experiment 5 in 1925. The male palms represented are: A, Canariensis No. 1; B, Mosque; C, Government No. 1; and D, Fard No. 4

get such proof would require many years and would even then be subject to question because of the absence of genetically pure strains of pollen.

#### OFF-TYPE SEEDS

Seeds entirely normal in appearance but showing marked variation from the average type in any one pollination naturally suggest

foreign pollen. On the other hand, seeds of abnormal appearance sometimes occur under such conditions as to make it very likely that some physiological disturbance in the development of the embryo and endosperm is responsible rather than another pollen. In a few of the Fard pollinations, both in 1925 and in 1926, there occurred occasionally an extra large seed, generally very irregular in shape, with a long, tapering apex more or less curved to one side; light colored; ventral surface rather flat and a ventral furrow which instead of a normal closure appeared split throughout its entire length. The fruits containing such seeds were also larger than the average and remained immature much longer, rarely ripening normally. This accounts for the delayed ripening of 1 date from Fard No. 4 pollen in experiment 4, 1 in experiment 5, 1 in experiment 9, and 2 in experiment 8. Only in two or three instances did any tendency toward this particular abnormality show in any of the other pollinations, though its occasional occurrence has been noted in various commercial gardens throughout the Coachella Valley.

Here also should be mentioned another phenomenon—the occasional appearance of one or more longitudinal ridges or “wings” on the seed. These ridges if pronounced usually produce a longitudinal depression in the flesh immediately above, which is more or less prominent until the dates begin to soften. Such seeds seem to occur more commonly with pollen from some palms than with that from others, but apparently may be found now and then with any pollen. Out of the *canariensis* pollinations there were one seed in 1925 and two in 1926 which developed a longitudinal ridge but which otherwise were unmistakably typical seeds produced by *canariensis* pollen.

#### DEVELOPMENT AND RIPENING OF FRUIT

A good setting of fruit was obtained in all of the pollinations except the *canariensis*, which was below normal. In most of the experiments the Mosque pollen gave a little better setting than the Fard No. 4, but the difference would seldom have attracted attention in a commercial garden.

As early as the first week in June, 1925, it was evident that the dates from the *canariensis* pollinations were smaller than those from the others, and even before this the smaller size of the unpollinated dates could be observed.

By the middle of July the bulk of the Deglet Noor dates in the Coachella Valley, where these tests were initiated, have usually reached about their maximum size and begin to change in color from green to a bright coral red, commonly ranging around carnelian red (R. XIV),<sup>4</sup> which is characteristic of the preripe stage in this variety. During the period of color change every shade from pure green to bright red may be found at the same time on the same cluster and often on the same fruit. However, differences in the rate at which the fruit from the various pollinations took on the red color were very apparent. In fact, the earlier coloring of the fruit from pollinations with Fard No. 4 was one of the striking features of these tests. Although distinguishable in all of the experiments, it was especially obvious where the pollinations were side by side on the same bunch.

<sup>4</sup> All color references are from RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912. Published by the author. The color cited, “carnelian red,” is quoted from Ridgway's plate 14, being indicated by R. XIV.

These differences in coloring were found to be followed by subsequent differences in ripening, so in the 1926 experiments at the Indio station detailed notes were made at a time approximately in the middle portion of the period of color change. These observations are given in Table 3. Differences in the rate of ripening of the fruit are shown in Tables 1, 2, 4, and 5.

TABLE 1.—*Ripening of the fruit of Deglet Noor palms in experiments 1, 2, 3, and 7 during the year 1925, at Indio, Calif., including the percentage ripe by September 30 and that damaged by rain October 4 and 5*

Experiment	On Deglet Noor palm No.	Pollen used	Date of pollination	Weekly pickings (ounces)																	Total	Percentage of fruit ripe Sept. 30	Percentage of fruit damaged by rain Oct. 4 and 5
				September							October					November							
				Aug. 27	3	9	17	23	30	7	15	22	29	5	12	19	26						
No. 1.	2-8-1	Mosque.....	Mar. 2	18	30	34	111	114	150	132	44	74	52	14				773	59.1	3.1			
		Fard No. 4.....	Feb. 23	69	55	51	124	113	40	12								464	97.4	.8			
		Canariensis No. 1.....	Feb. 25	24	41	25	66	32	39	60	6	6	2	2				303	74.9	9.5			
		Deglet Noor R-6.....	Feb. 27	77	53	67	107	84	117	98	36	51	4					694	72.7	4.3			
No. 2.	2-9-1	Mosque.....	Feb. 25	38	66	132	90	38	33	57							454	87.4	4.6				
		Fard No. 4.....	Feb. 26	55	46	48	152	109	78	42							530	92.0	3.4				
		Canariensis No. 1.....	Feb. 24	24	35	38	88	56	66	92	40	18	14	4			479	64.0	14.4				
		Deglet Noor R-6.....	Feb. 26	35	36	49	117	108	135	172	19						671	71.5	9.7				
No. 3.	2-5-7	Mosque.....	Mar. 23				30	38	42	34	17	20	20	6			207	53.1	.9				
		Fard No. 4.....	Mar. 19				80	52	92	67	15	14	3				323	69.3	1.2				
		Government No. 1.....	Mar. 12				58	60	84	109	36	55	26	5			433	46.6	3.2				
		Huey.....	Mar. 30				9	19	32	41	12	20	16	7			156	38.4	4.5				
No. 4.	2-9-1	Canariensis No. 1.....	Apr. 10				2	8	19	5	8	2	4	8	1	1	58	17.2	5.0				
		Mosque.....	Mar. 21		25	93	73	119	199	51	29	33	20				645	48.5	21.5				
		Fard No. 4.....	Mar. 22		41	113	62	84	62								362	82.8	4.9				
		Huey.....	Mar. 26		16	28	26	58	116	4							248	51.6	29.4				

\* Fruit damaged by the rain on October 4 and 5 is included in the picking of October 7.

TABLE 2.—*Relative maturity of dates in experiments 4, 5, and 6 in 1925 at Indio, Calif.*

[The several pollens were applied to different strands on the same inflorescence in each experiment]

Experiment	On Deglect Noor palm No.	Pollen used	Date of pollination	Total number of fruits	August 18			August 27			September 16		
					Number of fruits		Fruits ripe and partly ripe (per cent)	Number of fruits		Fruits ripe and partly ripe (per cent)	Number of fruits		Fruits ripe and partly ripe (per cent)
					Ripe	Partly ripe		Ripe	Partly ripe		Ripe	Partly ripe	
No. 4	2-8-1	Mosque. Fard No. 4. Government No. 1. Canariensis No. 1.	Mar. 10	104 57 124 77	0 18 0 2	7 5 5 0	6.7 40.3 4.0 2.6	9 37 12 5	8 10 5 2	16.3 82.5 13.7 9.1			
No. 5	2-9-1	Mosque. Fard No. 4. Government No. 1. Canariensis No. 1.	Mar. 14	127 79 122 49	0 4 0 0	0 0 0 0	5.1 — — —	3 11 4 0	0 2 3 0	2.4 16.5 5.7 0			
No. 6	2-5-7	Mosque. Fard No. 4. Government No. 1. Canariensis No. 1.	Mar. 10	69 84 124 63	0 8 0 0	0 10 0 0	— 21.4 — —	— — — —	— — — —	— — — —	43 84 80 30	0 0 0 0	82.3 100 70.2 47.6

TABLE 3.—Differences in coloring of dates in experimental pollinations during the period of transition from green to characteristic preripe color in 1926 at Indio, Calif.

Certain applications of pollen are marked with an asterisk (\*) indicating that the protective bags were partially unsealed by rain on April 5

Experiment No., variety used, and date of observation	Date of pollina- tion	Pollen used	Total num- ber of fruits	Fruits showing reddish tints		Remarks (relating to color)
				Num- ber	Per cent	
No. 1, on Deglet Noor 2-8-1, July 20.	Feb. 11	Mosque.....	123	66	54	Well advanced.
	Feb. 14	Canariensis No. 2....	89	26	29	Faint.
	Feb. 16	Deglet Noor, N-12....	100	71	71	Comparable to Fard
	Feb. 18	Fard No. 4.....	107	71	66	Well advanced.
	Feb. 24	(No pollen).....				
	Mar. 2	Fard, FNJ-S.....	89	34	38	Faint.
	Mar. 6	Fard, FNJ-N.....	80	37	46	Do.
	Mar. 12	*Menakher No. 1....	112	5	4	Do.
	Mar. 13	*Maktum No. 8.....	99	11	11	Do.
No. 2a, on Deglet Noor 2-9-1, July 19.	Mar. 24	*Deglet Noor, R-6....	104	1	1	Very faint.
	Feb. 16	Mosque.....	78	22	28	Faint.
	do.	Canariensis No. 3....	27	8	30	Do.
	Feb. 17	(No pollen).....				
	Feb. 17	Fard No. 4.....	73	44	61	Intermediate.
	Feb. 20	Maktum No. 5.....	75	25	33	Faint.
	Mar. 2	Fard, FNJ-S.....	96	94	98	Well advanced.
	Mar. 8	Fard, FNJ-N.....	80	78	98	Do.
	Mar. 10	Deglet Noor, N-10....	82	32	39	Intermediate.
No. 2b, on Deglet Noor 2-9-1, July 27.	Mar. 15	*Mosque.....	122	8	7	Very faint.
	do.	*Fard No. 4.....	96	51	53	Faint.
	Mar. 20	*Deglet Noor, N-18....	95	20	21	Very faint.
No. 3, on Deglet Noor 2-5-7, July 27.	Mar. 1	Canariensis No. 3....	73	44	60	Faint.
	Mar. 9	*Mosque.....	98	19	19	Do.
	Mar. 10	*Fard No. 4.....	71	43	61	Do.
	Mar. 15	*Government No. 1....	70	19	27	Very faint.
	Mar. 22	*(No pollen).....				
	Mar. 22	*(No pollen).....				
No. 4, on Deglet Noor 2-8-1, July 16.	Feb. 11	(Mosque.....	128	44	34	Intermediate.
		Fard No. 4.....	63	7	75	Well advanced
		Canariensis No. 2....	94	16	17	Very faint.
		Deglet Noor, N-12....	98	42	42	Intermediate.
No. 5, on Deglet Noor 2-8-1, July 27.	Mar. 20	(Mosque.....	147	25	17	Faint.
		Fard No. 4.....	89	83	93	Faint, few intermediate.
		Saidy, No. 13.....	117	49	42	Faint.
		Deglet Noor, N-18....	105	27	26	Do.
No. 6, on Deglet Noor 2-8-1, July 27.	Mar. 23	(Mosque.....	98	20	20	Faint.
		Fard No. 4.....	84	55	65	Faint, few intermediate.
		Deglet Noor, R-1....	109	45	41	Faint.
		Deglet Noor, R-6....	127	9	7	Very faint.
No. 7, on Deglet Noor 2-9-1, July 27.	Feb. 26	(Fard No. 4.....	94	91	97	Well advanced.
		Fard, FNJ-S.....	57	55	96	Do.
		Canariensis No. 2....	27	18	67	Faint.
		Maktum No. 5.....	80	63	79	Intermediate.
		Maktum No. 6.....	85	81	95	A little behind Maktum No. 7.
		Maktum No. 7.....	91	87	96	Well advanced.
No. 8, on Deglet Noor 2-9-1, July 27.	Mar. 8	(Mosque.....	69	24	35	Less advanced than Fard.
		Fard No. 4.....	83	72	87	Well advanced.
		Menakher No. 1....	68	35	51	Intermediate.
		Thoori No. 20.....	59	33	56	Do.
		Saidy No. 25.....	64	35	55	Do.
		Fard, FNJ-N.....	83	81	97	Well advanced.
		Deglet Noor, N-9....	82	46	56	Intermediate.
		Ascherasi.....	89	54	61	Do.
No. 9, on Deglet Noor 2-5-7, July 16.	Feb. 25	(Mosque.....	84	12	14	Faint.
		Fard No. 4.....	45	17	38	Intermediate.
		Fard, FNJ-S.....	44	19	43	Do.
		Canariensis No. 2....	57	4	7	Very faint.
		Deglet Noor, N-9....	81	24	30	Faint.
No. 10, on Deglet Noor 2-8-2, July 27.	Mar. 8	(Ascherasi.....	86	82	95	Intermediate.
		Deglet Noor, N-9....	88	70	80	Do.
		Thoori No. 20.....	56	44	79	Do.

TABLE 3.—*Differences in coloring of dates in experimental pollinations during the period of transition from green to characteristic preripe color in 1926 at Indio, Calif.—Continued*

Experiment No., variety used, and date of observation	Date of pollina- tion	Pollen used	Total num- ber of fruits	Fruits showing reddish tints		Remarks (relating to color)
				Num- ber	Per cent	
No. 11, on Deglet Noor 2-9-2, July 27.	} Mar. 9	Fard No. 4.....	45	40	89	Intermediate.
		Fard, FNJ-N.....	57	53	93	Do.
		Saidy No. 25.....	92	62	67	Do.
		Deglet Noor, N-9....	76	37	49	Faint.
No. 12, on Deglet Noor 2-6-3, July 27.	} Mar. 9	Maktum No. 5.....	99	21	21	Faint.
		Maktum No. 6.....	101	29	29	Do.
		Thoory No. 20.....	102	13	13	Very faint.
No. 13, on Deglet Noor seedling No. 6, July 19.	} Mar. 10	Mosque.....	52	25	48	Less advanced than Fard.
		Fard No. 4.....	87	87	100	Well advanced.
		Canariensis No. 2....	99	90	91	Less advanced than Fard.
No. 14, on Rhars, 1-8-6, July 13.	} Feb. 15	Mosque.....	23	0	0	
		Fard No. 4.....	16	9	56	
		Canariensis No. 2....	50	19	38	Preripe color, yellow.
No. 15, on Khadrawy, 2-1-8, July 27.	} Mar. 24	Mosque.....	38	8	21	
		Fard No. 4.....	39	21	54	Preripe color, yellow.
No. 16, on Maktum, 2-7- 7, July 27.	} Mar. 13	Mosque.....	57	10	18	
		Fard No. 4.....	93	57	61	
		Maktum No. 5.....	60	21	35	Preripe color, yellow.
No. 17, on Deglet Noor 2-4-4, Aug. 19.	} Apr. 12	Mosque.....	56	45	80	Faint.
		Fard RB No. 1.....	69	67	97	Intermediate.
No. 18, on Deglet Noor 2-4-4, Aug. 19.	} Apr. 14	Mosque.....	45	37	82	Faint.
		Fard A-21-2-32....	50	48	96	Intermediate.

\* Two strands only, one having been broken off.



TABLE 4.—Progressive ripening of dates resulting from experimental pollinations in 1936 at Indio, Calif.

Experiment No. and variety used	Date of pollination	Pollen used	Total number of fruits	Aug. 17		Aug. 18		Aug. 27		Sept. 3		Sept. 13		Sept. 14		Sept. 30	
				Number of fruits		Number of fruits		Number of fruits		Number of fruits		Number of fruits		Number of fruits		Number of fruits	
				Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)	Ripe	Fruits ripe and partly ripe (per cent)
No. 1, Deglet Noor 2-8-1----	Feb. 11	Mosque.....	115	24	27	45	71	99	89	109	6	100				81	100
	Feb. 14	Canariensis No. 2.....	85	16	33	21	55	59	74	71	4	88					
	Feb. 16	Deglet Noor N-12.....	95	36	41	77	84	92	98	95		100					
	Feb. 18	Fard No. 4.....	106	30	37	75	83	99	93	104	2	100					
	Mar. 2	Fard FNI-S.....	87	24	31	65	64	80	93	85	2	100					
	Mar. 6	Fard FNJ-N.....	79	19	30	65	2	85	71	2	92	78	0	99		79	100
	Mar. 12	Menakher No. 1.....	109	4	2	24	12	33	39	7	42	80	13	85		109	100
	Mar. 13	Maktum No. 5.....	99	10	1	29	6	35	51	2	54	80	4	85		99	100
	Mar. 24	Deglet Noor H-6.....	96	2	0	5	7	13	13	8	22	48	8	58		96	90
	Feb. 16	Mosque.....	76	9	5	29	11	49	51	5	74	71	3	97		76	100
No. 2a, Deglet Noor 2-9-1----	Feb. 17	Canariensis No. 3.....	23	3	42	62	77	83	83	1	92	100				26	100
	Feb. 17	Fard No. 4.....	70	23	11	62	77	83	83	1	92	100				75	100
	Feb. 20	Maktum No. 5.....	73	11	8	33	12	63	60	3	84	72	2	90			
	Mar. 2	Fard FNI-S.....	94	20	8	87	4	97	92	0	98	93	0	99		91	100
No. 2b, Deglet Noor 2-9-1----	Mar. 8	Fard FNI-N.....	70	20	8	60	5	82	72	2	94	70	0	100			
	Mar. 10	Deglet Noor N-10.....	79	11	5	24	4	35	37	5	53	58	7	82		70	100
	Mar. 15	Mosque.....	120	0	2	15	6	18	26	10	30	55	19	62		120	100
	do.....	Fard No. 4.....	65	11	6	36	4	61	26	21	86	93	2	100			
	Mar. 20	Deglet Noor N-18.....	94	3	0	11	5	17	25	7	35	49	8	61		87	96
	Mar. 20	Deglet Noor N-18.....	94	3	0	11	5	17	25	7	35	49	8	61		87	96
No. 3, Deglet Noor 2-5-7----	Mar. 1	Canariensis No. 3.....	70	3	1	8	8	23	15	6	30	37	8	64		62	90
	Mar. 9	Mosque.....	90	0	0	0	0	3	4	8	11	37	7	49		66	89
	Mar. 10	Fard No. 4.....	71	1	3	18	7	35	30	3	46	38	4	59		69	99
	Mar. 15	Government No. 1.....	68	0	0	0	0	5	8	11	5	24	5	46		65	97
	Mar. 15	Government No. 1.....	68	0	0	0	0	5	8	11	5	24	5	46		65	97
No. 4, Deglet Noor 2-8-1----	Feb. 11	Mosque.....	122	57	5	113	4	96	122	0	100					80	100
	Feb. 11	Fard No. 4.....	90	50	7	59	0	93	50	0	98	60					
	Feb. 11	Canariensis No. 2.....	91	13	6	34	6	44	41	2	47	63	2	71		80	100

TABLE 4.—Progressive ripening of dates resulting from experimental pollinations in 1926 at Indio, Calif.—Continued

Experiment No. and variety used	Date of pollination	Pollen used	Total number of fruits	Aug. 17		Aug. 18		Aug. 27		Sept. 3		Sept. 13		Sept. 14		Sept. 30			
				Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)	Number of fruits	Fruits ripe and partly ripe (per cent)
No. 5, Deglet Noor 2-8-1-----	Mar. 20	Mosque.....	146	3	1	3	15	6	14	34	4	26	51	16	46	142	2	99	
		Fard No. 4.....	86	12	4	19	37	6	50	56	3	70	77	2	92	85	0	99	
		Sady No. 13.....	116	8	3	9	28	6	20	36	3	34	58	17	65	116	4	100	
No. 6, Deglet Noor 2-8-1-----	Mar. 23	Deglet Noor N-18.....	102	4	0	4	11	2	13	23	3	25	51	7	57	93	4	95	
		Mosque.....	98	0	2	2	9	6	15	19	3	22	40	7	48	87	6	95	
		Fard No. 4.....	83	8	7	18	27	12	47	40	7	57	71	6	93	106	1	100	
No. 7, Deglet Noor 2-9-1-----	Feb. 26	Deglet Noor R-1.....	107	3	3	6	19	10	27	31	7	36	53	14	63	104	9	93	
		Deglet Noor R-6.....	122	1	0	1	2	1	2	5	0	4	20	11	25	94	94	9	100
		Fard No. 4.....	94	21	7	30	71	13	89	87	4	97	92	0	98	94	94	100	
No. 8, Deglet Noor 2-9-1-----	Mar. 8	Fard FNJ-S.....	55	15	8	42	45	6	93	53	0	96	54	1	98	55	55	100	
		Canariensis No. 2.....	25	0	1	4	5	0	20	5	2	28	12	1	52	25	25	100	
		Makthum No. 5.....	79	8	4	15	35	7	53	49	7	71	75	2	97	79	79	100	
No. 9, Deglet Noor 2-5-7-----	Feb. 25	Makthum No. 6.....	53	10	5	18	52	12	77	70	7	93	82	0	99	83	83	100	
		Makthum No. 7.....	92	7	11	20	60	14	80	83	4	95	89	1	98	92	92	100	
		Mosque.....	67	2	2	6	17	7	36	30	2	48	44	3	70	62	2	96	
No. 8, Deglet Noor 2-9-1-----	Mar. 8	Fard No. 4.....	82	15	3	22	37	3	49	43	4	57	56	5	82	79	1	98	
		Menaker No. 1.....	67	0	1	1	14	10	36	29	12	61	50	2	82	63	0	94	
		Theory No. 20.....	60	5	3	13	20	6	43	37	12	82	51	2	88	60	60	100	
No. 8, Deglet Noor 2-9-1-----	Mar. 8	Sady No. 25.....	58	0	4	7	20	7	47	22	10	55	47	7	93	58	58	100	
		Fard FNJ-N.....	82	10	4	17	44	13	70	72	1	80	80	1	90	81	1	100	
		Deglet Noor N-9.....	83	3	3	7	25	7	39	35	2	45	54	6	72	83	83	100	
No. 9, Deglet Noor 2-5-7-----	Feb. 25	Ascherasi.....	90	6	8	15	31	23	60	55	14	77	83	5	98	89	1	100	
		Mosque.....	82	5	9	17	39	9	59	55	4	72	73	6	96	81	1	100	
		Fard No. 4.....	44	9	12	48	33	5	86	41	1	95	43	0	98	43	0	98	
No. 9, Deglet Noor 2-5-7-----	Feb. 25	Fard FNJ-S.....	42	9	9	43	32	4	86	39	2	97	49	0	98	41	41	98	
		Canariensis No. 2.....	57	0	0	0	21	4	44	27	1	49	36	10	81	57	57	100	
		Deglet Noor N-9.....	81	4	11	19	36	5	51	44	4	59	72	4	94	81	81	100	

No 10, Deglet Noor 2-8-2.....	Mar. 8	Ascherasi { Deglet Noor N-4 { Theory No. 20.....	83 13 15 34	88 70 6	99 6	100	88	100
			88 12 6 20	89 62 10	99 78 4	100	88	100
			55 14 6 36	82 51 3	98 55	100	42	100
No. 11, Deglet Noor 2-4-2.....	Mar. 9	{ Fard No. 4.....	42 10 8 64	88 38 0	90 40 0	95	42	100
		{ Fard No. 5.....	52 12 7 44	98 56 1	100 100	98	91	0
		{ Sady No. 25.....	52 12 7 44	79 82 2	91 90 0	98	91	0
		{ Deglet Noor N-9.....	76 2 6 11	67 64 7	93 73 2	99	76	100
No. 12, Deglet Noor 2-6-3.....	do	{ Maktum No. 5.....	95 2 0 2	7 17 8	26 41 9	53	94	100
		{ Maktum No. 6.....	83 2 2 4	11 19 6	27 48 12	65	92	0
		{ Theory No. 20.....	89 1 1 2	8 20 4	24 44 10	63	99	100
No. 13, Seedling 20-6.....	Mar. 10	{ Mosque.....	52 9 2 21	36 25 6	60 42 2	85	52	100
		{ Fard No. 4.....	76 16 6 29	57 52 5	75 69 5	97	76	100
		{ Canarensis No. 2.....	98 3 3 6	9 20 1	21 30 8	39	59	68
No. 14, Rhars 1-8-6 <sup>a</sup> .....								
No. 15, Khadrawy 2-1-8.....	Mar. 24	{ Mosque.....	36 5 2 19					
		{ Fard No. 4.....	39 0 0 17	0 5	9 10 13	40	45	3
		{ Mosque <sup>b</sup> .....	57 2 6 9	35 26	66 72 14	92	48	100
No. 16, Maktum 2-7-7.....	Mar. 13	{ Fard No. 4.....	93 0 0 3	18 0	30 34 5	65	58	1
		{ Maktum No. 5.....						
No. 17, Deglet Noor 2-4-4.....	Apr. 12	{ Mosque.....	56 2 2 2				0 3	5 20 19 86
		{ Fard AB No. 1.....	69 2 2 2				2 22	35 61 6 97
No. 18, Deglet Noor 2-4-4.....	Apr. 14	{ Mosque.....	45 2 2 2				1 2	7 21 15 80
		{ Fard A-21-2-32.....	47 2 2 2				12 12	51 43 3 98

<sup>a</sup> All the dates ripened before the first notes on maturity were made.<sup>b</sup> Two strands only.

TABLE 5.—*Relative maturity of dates resulting from experimental pollinations in the Salt River Valley, Ariz., in 1926*

[Experiments 1-7 were at the Tempe Date Garden, and experiments 8 and 9 were at the garden of the Arizona Orchards Company. Pollens from different sources were applied on the same bunch in each experiment except 1 and 6.]

Experiment No., variety used, and date of observation	Pollen used	Date of pollination	Total number of fruits	Number of fruits		Fruits ripe and partly ripe (per cent)
				Ripe	Partly ripe	
No. 1, Deglet Noor No. 1, Sept. 20.	Mosque.....	Mar. 30	93	27	25	56
	Fard No. 4.....	do.....	47	31	13	94
No. 2, Deglet Noor No. 1, Sept. 20.	Mosque.....	do.....	68	22	12	50
	Fard No. 4.....	do.....	33	18	9	82
No. 3, Deglet Noor No. 1, Sept. 20.	Mosque.....	Mar. 31	55	17	15	58
	Fard No. 4.....	do.....	59	49	4	90
No. 4, Deglet Noor No. 1, Sept. 20.	Mosque.....	Mar. 30	45	13	15	62
	Fard No. 4.....	do.....	68	51	14	96
	Fard FNJ-S.....	do.....	71	43	13	79
	Fard FNJ-N.....	do.....	18	16	1	94
	Mosque.....	do.....	44	17	8	57
No. 5, Deglet Noor No. 2, Sept. 21.	Fard No. 4.....	do.....	70	39	18	82
	Fard FNJ-N.....	do.....	61	34	20	89
	Fard FNJ-S.....	do.....	56	36	9	80
	Mosque.....	do.....	50	11	13	48
No. 6, Deglet Noor No. 2, Sept. 21.	Fard No. 4.....	do.....	64	20	29	77
	Fard FNJ-S.....	Mar. 31	44	17	12	66
	Mosque.....	Mar. 30	57	10	8	32
No. 7, Deglet Noor No. 2, Sept. 21.	Fard No. 4.....	do.....	45	18	14	71
No. 8, Deglet Noor, Sept. 22.	Mosque.....	Mar. 29	51	0	0	0
	Fard No. 4.....	do.....	50	13	5	36
No. 9, Iteema, Sept. 22.....	Mosque.....	do.....	19	0	2	11
	Fard No. 4.....	do.....	28	9	1	36

\* Two strands.

† One strand.

‡ Six of these had already been picked, but the calyxes remaining left no doubt about it.

In experiments 1, 2, 3, and 7 in 1925, in which each of the several pollens was applied to an entire inflorescence, weekly pickings of ripe fruit were made. (Table 1.) In experiment 8 no record of the ripening was kept, but it was very apparent that the fruit from the Fard No. 4 pollination matured earlier than that from Mosque. In all of the other experiments the record is based on the total number of dates on three typical strands from each pollination—all that were pollinated where several pollens were applied to the same inflorescence—and each observation includes the actual number of dates that were ripe or had already ripened and the number that were partly ripe.

#### DAMAGE FROM RAIN, FUNGI, AND LOW HUMIDITY

In 1925 the normal seasonal ripening was interrupted on October 4 and 5 by a heavy rain which caused more or less damage to fruit in the preripe stage just before the final color change from red to amber. No appreciable injury was done except in experiments 1, 2, 3, and 7. The damaged fruit, which normally would have ripened some weeks later, was picked within a few days and weighed separately, but entered in the total production. The weights were found to be about 20 per cent heavier than for an equal number of ripe dates, but this was probably more than offset by the proportion of fruits which fell

to the ground before they could be picked. Since the fruit affected had not begun to ripen, the relative damage from the rain affords a partial index to the maturity of the dates from the various pollinations. Of course the position and exposure of the different bunches also had some influence in this connection, but, as is shown in the last column of Table 1, the damage to the fruit from Fard No. 4 pollen was very low in every instance.

Following in the wake of the rain a week or so later, a period of cloudy weather accompanied by high humidity fostered a fungus which caused the development of soft rot, dates in the preripe stage having been chiefly affected. No account of the loss from this source could well be taken in the total production, as much of the rotting fruit fell to the ground from day to day and no attempt was made to pick any of it. However, the ripening had progressed so far that the loss was negligible except in experiment 3, and even in this the fruit from Fard No. 4, having nearly all ripened, was practically unaffected, though the loss of that from Mosque was estimated roughly at about 20 per cent and that from Canariensis No. 1 at about 50 per cent. Because of this unrecorded loss of fruit which would have ripened later, it is evident that in a normal season the actual percentage of early ripening fruit would probably have been less for all of these pollinations except the Fard No. 4.

In the Deglet Noor variety a small percentage of premature shriveling is not uncommon as the fruit ripens. In 1926 the season was earlier than usual, and the low humidity prevailing as the dates began to ripen produced an unusual proportion of shriveling throughout the Coachella Valley. In a few of the experiments at the Indio station as much as 5 to 10 per cent of the fruit was affected, but taking the experiments as a whole, fortunately very little appeared. In recording the ripening, fruit which shriveled before the final color change was discounted and eliminated from the total, but otherwise it was counted as ripe. Since there was no consistent tendency for more of this shriveling to appear in the fruit from one pollen than in that from another, the reliability of a series of experiments would not be appreciably affected.

#### FACTORS OTHER THAN POLLEN AFFECTING TIME OF RIPENING

Of the factors other than pollen which possibly may affect the time of ripening of the fruit, many, such as fertilizer, irrigation, and pruning, have not yet been subjected to intensive study, but they would have no direct bearing on the evidence presented here because they would affect equally all of the fruit on an individual palm.

In considering the ripening data, the effect of the earliness or lateness of the season should be borne in mind. Dates which begin to mature in the extreme heat of late summer ripen much more rapidly than those which mature later in the cooler fall weather. Hence, if the ripening begins only a week earlier, the last of the crop may be off the palms as much as a month earlier, the tapering effect of cooler weather being cut short. This is what occurred in 1926. Consequently, an early season tends to lessen differences in the time of ripening due to pollen, whereas a late season accentuates them.

Since the normal duration of the flowering season for an individual palm of the Deglet Noor variety is about six or seven weeks, rather

longer than that of most other varieties, it is important to consider the differences in ripening which may be due to differences in the time of blooming. To determine whether such differences exist, a record was kept in 1925 of the inflorescences on four Deglet Noor palms in commercial bearing, the same pollen being used on all of the flower clusters on each palm. There was an average difference of six weeks and four days between the opening of the first and the last spathes. Weekly pickings of ripe fruit from each cluster were made, from which it appeared that there was an average difference of approximately three weeks between the initial ripening of the first and the last clusters.

A comparison of the time of ripening of the fruit with respect to the time of pollination in Tables 1, 3, and 4 affords additional data. For instance, in Table 4, while the pollination with Mosque in experiment 2a was a month earlier than that with Mosque in experiment 2b, the actual difference in the time of ripening of the fruit was only about 12 days. Approximately the same difference is apparent in the results obtained with Fard No. 4 in these two experiments. On the other hand, the fruit from the pollination with Fard No. 4 in experiment 2b ripened very nearly on a par with that from the Mosque pollination in experiment 2a, though there was a month difference in the time of pollination.

Here again other factors cause variation. From an inspection of date gardens throughout the Coachella Valley it was evident that the differences in the time of ripening between the first and the last clusters on individual palms was much less in warmer localities where all the ripening was earlier, being in some cases almost negligible from a casual inspection. On the other hand, in localities where all the ripening was later, the differences were more in contrast than they were at the Indio station, in some instances the first clusters being practically stripped of fruit before any pickings were made from the last ones.

#### COMPARISON OF SIZES OF FRUITS AND SEEDS

In 1925 measurements were made in experiments 1, 2, 3, 7, and 8 of the fruits and seeds of 100 ripe dates, taken at random from not less than four pickings between September 1 and October 15. In experiments 4, 5, and 6 fewer than 100 dates were available, as indicated in the tabulations, all of which were measured at the same time after all the fruit had ripened. These measurements are given in Tables 6, 7, and 8. Similar measurements for the 1926 experiments are given in Tables 8 to 11, which, except as indicated, represent 30 dates from each pollination after all of the fruit had been picked. Each 30 were selected at random except that no abnormal fruits or seeds were measured. Those not measured were mostly deformed dates or those where two developed in the same calyx; in the seed, those imperfectly developed, as indicated by a small formless endosperm or by an exaggerated development all out of proportion. No doubtful specimens were excluded.



TABLE 8.—*Breadths of date fruits and seeds in the 1925 experiments at Indio, Calif.*

Experiment	Number measured	Pollen used	Fruit measurements (millimeters) and frequencies												Seed* measurements (millimeters and frequencies)											
			16	17	18	19	20	21	22	23	24	Mean	5	6	7	8	9	10	11	Mean						
No. 1.	100	Mosque.....			2	5	20	58	12	3		19.82			1	48	50	1		8.51						
		Fard No. 4.....			3	11	41	36	8	1		19.38			5	64	29	1	1	7.29						
		Deglet Noor R-6.....			10	32	41	16	1			18.06			1	42	53	4		7.60						
		Canariensis No. 1.....	1		8	29	44	14	3	1		18.75	1	69	28	1	1			6.32						
No. 2.	100	Mosque.....			2	16	56	22	4			20.10			4	40	52	4		8.56						
		Fard No. 4.....			6	29	32	10	3			19.75			4	53	39	4		7.43						
		Deglet Noor R-6.....			1	9	32	47	10	1		19.59			1	40	57	2		7.60						
		Canariensis No. 1.....			1	18	44	28	7	2		19.28	3	65	29	1	2			6.34						
No. 3.	100	Mosque.....			6	38	53	3				19.53			1	32	64	3		8.69						
		Fard No. 4.....			8	36	46	10				18.58			1	31	61			7.74						
		Government No. 1.....			17	57	25	1				19.10			4	67	29			8.25						
		Canariensis No. 1.....			7	32	44	17				18.71	1	19	75	5				6.84						
No. 4.	30	Huey.....			1	11	63	23	2			19.14			6	72	22			8.16						
		Mosque.....					7	7	12	3	1	21.46			1	4	12	11	2	8.30						
		Fard No. 4.....					3	16	6	5		20.43	1	2	23	3	1			7.03						
		Government No. 1.....					5	11	9	5		21.46			3	21	6			7.10						
No. 5.	40	Canariensis No. 1.....			1	3	16	6	4			20.30			3	23	3	1		7.06						
		Mosque.....					8	24	7	1		20.02			2	28	9	1		8.22						
		Fard No. 4.....			1	9	18	11	1			20.05			20	19	1			7.52						
		Government No. 1.....					8	21	11			20.07			4	28	8			8.10						
No. 6.	45	Canariensis.....			1	4	17	16	2			19.53	14	26						6.65						
		Mosque.....					1	10	25	9		19.93			1	8	27	8	1	9.00						
		Fard No. 4.....					7	24	14			19.15			16	29				7.64						
		Government No. 1.....					4	21	15	5		19.46			3	30	10	2		8.24						
No. 7.	100	Canariensis No. 1.....			1	14	19	11				18.88	5	34	6					7.02						
		Mosque.....					2	19	59	18	1	20.00			6	55	37	2		8.35						
		Fard No. 4.....			1	9	32	55	3			19.50	3	74	23					7.20						
		Huey.....			1	1	21	48	26	6		21.12			16	67	17			8.01						
No. 8.	100	Mosque.....					21	35	28	15	1	21.40			16	72	12			7.96						
		Fard No. 4.....			2	7	49	30	12			20.43			4	59	37			7.33						

\* Measured at point of greatest diameter across and at right angles to the seed furrow.

TABLE 9.—*Length of date fruits in the 1926 experiments at Indio, Calif.*

[Measurements of 30 fruits were made except where a smaller number is indicated in parentheses]

Experiment	Pollen used	Measurements (millimeters) and frequencies																										
		29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	Mean				
No. 1.	Mosque.....												1	5	2	12	8			2					42.0			
	Fard No. 4.....									2	5	13	2	3	5										38.5			
	Canariensis No. 2.....									3	5	11	6	3	2										38.2			
	Deglet Noor N-12.....												4	4	9	8	3	1	1						41.3			
	Fard FNJ-S.....								1		5	6	4	6	4	2	1								40.2			
	Fard FNJ-N.....									2	3	3	7	9	6	2	1								40.8			
	Menakher No. 1.....										1	1	6	5	8	3	4	2							41.8			
	Maktum No. 5.....										2	6	9	6	5	2									40.4			
No. 2a.	Deglet Noor R-6.....											1	6	11	4	1	4	2	1						41.8			
	Mosque.....												1		1	4	6	5	10	3					44.8			
	Fard No. 4.....										1			4	6	7	7	3	2						42.0			
	Canariensis No. 3.....									1	2	3	8	9	4	2	1								39.6			
	Maktum No. 5.....												2	5	4	6	10	2	1						42.9			
	Fard FNJ-S.....									1	2	8	1	4	8	5	1								40.8			
	Fard FNJ-N.....											1	4	7	11	6	1								41.7			
	Deglet Noor N-10.....									1	2	2	6	4	5	5	4	1							41.4			
No. 2b.	Mosque.....											1	1	9	11	10	5	3							41.5			
	Fard No. 4.....										3	5	12	5	4	1									40.2			
	Deglet Noor N-18.....												2	2	6	5	11	3	1						43.1			
	Mosque.....												1	6	10	7		3	3						41.7			
	Fard No. 4.....											4	5	9	7	2	3								39.2			
	Canariensis No. 3.....										3	2	5	6	7	5	2								38.1			
	Government No. 1.....											1	3	4	8	8	4	1	1						42.3			



TABLE 9.—Length of date fruits in the 1926 experiments at Indio, Calif.—Contd.

Experiment	Pollen used	Measurements (millimeters) and frequencies																												
		29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	Mean						
No. 4	Mosque													2	1	2	6	7	4	2	4	2			44.2					
	Fard No. 4									1	1	6	7	7	3	4	1								39.6					
	Canariensis No. 2					1	2	3	7	9	5			2	1										37.6					
	Deglet Noor N-12							1	1					4	8	8	6	2							40.5					
No. 5	Mosque											1	2	2	6	8	7	4							41.8					
	Fard No. 4							1	4	5	9	8	2	1											38.0					
	Saidy No. 13									1	1	3	6	11	6	2									40.7					
	Deglet Noor N-18									1	3	7	9	4	5	1									41.0					
No. 6	Mosque													5	9	6	5	3	2						41.9					
	Fard No. 4									1	3	4	9	5	3	4	1								39.5					
	Deglet Noor R-1										2	4	2	6	6	7	3								40.4					
	Deglet Noor R-6											1			2	5	7	6	5	3	1				43.5					
No. 7	Fard No. 4												4	6	7	8	3	1	1						41.2					
	Fard FNJ-S												3	2	9	10	2	4							41.6					
	Canariensis No. 2							1	1	4	5	2	1	1											37.9					
	(15)																													
No. 8	Maktum No. 5											1		4	4	10	3	3	4	1					42.3					
	Maktum No. 6													3	6	8	12	1							42.1					
	Maktum No. 7													1	5	4	13	2	2	3					41.9					
	Mosque														2	1	5	7	10	4		1			43.3					
No. 9	Fard No. 4							2	5		11	4	4	1	1	2									36.4					
	Menakher No. 1														5	4	3	6	6	3	2	1			43.9					
	Theory No. 20														3	3	9	8	5	2					43.5					
	Saidy No. 25														1	4	8	5	8	3	1				42.9					
No. 10	Fard FNJ-N												2	6	5	6	7	3	1						40.8					
	Deglet Noor N-9									1			3	6	2	4	6	4	3	1					41.9					
	Ascherasi												2	1	5	7	7	6	2						41.4					
	Mosque														1	7	5	7	7	2		1			42.8					
No. 11	Fard No. 4											1	3	5	7	3	6	4	1						40.6					
	Fard FNJ-S												9	3	13	4	1								39.5					
	Canariensis No. 2					1		1	2	8	9	4	4	1											37.8					
	Deglet Noor N-9											1	2	4	3	7	10	3							41.8					
No. 12	Ascherasi												1		5	7	8	6	3						41.7					
	Deglet Noor N-9													1	7	9	13								42.1					
	Theory No. 20														2		5	6	11	2	4				43.5					
	Fard No. 4														2	6	6	7	6	3		1	1		32.6					
No. 13	Fard FNJ-N														2	3	4	5	12	2	7	2			43.2					
	Saidy No. 25																								44.7					
	Deglet Noor N-9													1	4	7	8	4	4	2					45.0					
	Maktum No. 5											1	6	10	6	4	2	1							40.5					
No. 14	Maktum No. 6													2	6	5	10	5	1						41.0					
	Theory No. 20													2	1	4	5	5	8	2	1	2			42.0					
	Mosque														2	2		7	5	2	3	3	1	3	2	44.9				
	Fard No. 4											1			2	4	7	5	1	8	1	1			42.9					
No. 15	Canariensis No. 2											2	1	5	10	5	3	3	1						40.3					
	Mosque (15)																2		5	6	2				44.4					
	Fard No. 4 (7)														1		1	1	2		1	1			43.7					
	Canariensis No. 2											1	1	5	7	3	3								39.9					
No. 16	(20)																													
	Mosque (20)																													
	Fard No. 4 (20)																								33.1					
	Mosque (8)																								30.6					
No. 17	Fard No. 4 (20)																								33.1					
	Maktum No. 5 (20)																								31.8					
	Mosque																								32.0					
	Fard RB No. 1																								46.5					
No. 18	Mosque																								44.9					
	Fard A-21-2-32																								45.2					

TABLE 10.—Length of seeds of date fruits in the 1926 experiments at Indio, Calif.

[Measurements of 30 fruits were made except where a smaller number is indicated in parentheses]

Experiment	Pollen used	Measurements (millimeters) and frequencies																												Mean
		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31														
No 1	Mosque							2	7	6	8	3	2	1	1		25.6													
	Fard No. 4.				2	4	11	7	4		1	1					22.5													
	Canariensis No. 2			5	10	7	7	1									20.6													
	Deglet Noor N-12							6	12	8	2		2				24.5													
	Fard FNJ-S				1	3	13	6	5	2							22.6													
	Fard FNJ-N					3	7	4	10	3	1	1	1				23.5													
	Menakher No. 1							5	6	11	4	3	1				24.9													
No. 2a	Maktum No. 5							9	10	9	1	1					24.2													
	Deglet Noor R-6						1	2	6	12	7	1	1				25.0													
	Mosque							1	1	3	7	9	3	5	1		26.9													
	Fard No. 4.						1		8	14	5	2					24.9													
	Canariensis No. 3				3	7	9	6	4	1							22.1													
	Maktum No. 5.						2	1	2	6	4	10	3	1	1		26.1													
	Fard FNJ-S				1	1	2	9	11	4		2					23.7													
No. 2b	Fard FNJ-N						1	9	11	4	3	2					24.2													
	Deglet Noor N-10.					2	5	5	9	6	2	1					23.7													
	Mosque.						1	3	8	11	4	3					24.8													
	Fard No. 4.						1	12	9	2	6						23.0													
	Deglet Noor N-18.						1	1	6	7	9	3	1	1	1		25.5													
	Mosque.					2	3	5	8	7	4	1					23.0													
	Fard No. 4.							1	1	2	14		8	3	1		25.7													
No. 3	Canariensis No. 3.	1	1	7	9	9	1	2									20.1													
	Government No. 1							1	3	12	8	6					25.5													
	Mosque.							1	3	8	8	5	3	2			27.0													
	Fard No. 4.				1		6	5	13	4	1						22.5													
	Canariensis No. 2.	1	2	6	9	7	3	1	1								20.9													
	Deglet Noor N-12.						4	13	8	5							23.5													
	Mosque.							2	3	6	7	8	2	2			26.0													
No 5	Fard No. 4.			2	2	11	8	4	1	1		1					21.8													
	Saidy No. 13						1	6	8	7	8						24.5													
	Deglet Noor N-18.							11	8	7	3	1					24.2													
	Mosque.								4	3	11	8	4				26.2													
	Fard No. 4.						1	8	7	7							23.4													
	Deglet Noor R-1						2	3	3	12	6	3	1				24.0													
	Deglet Noor R-6.							1	2	4	14	6	1	1	1		26.1													
No. 6	Fard.						6	6	10	4	2	2					23.9													
	Fard FNJ-S.						1	1	4	6	13	2	3				24.6													
	Canariensis No. 2 (15).			1	1	2	8	2	1								21.8													
	Maktum No. 5.						3	5	4	8	6	4					24.7													
	Maktum No. 6.							5	7	5	10	1	2				25.0													
	Maktum No. 7.						1	7	10	3	5	4					24.5													
	Mosque.						2	2	4	10	6	5	1				25.2													
No. 8	Fard No. 4.				3	5	8	7	4	3							21.4													
	Menakher No. 1						1	1	2	6	8	8		3	1		26.1													
	Theory No. 20						1	2	5	8	9	5					25.2													
	Saidy No. 25.						1	3	9	7	9		1				25.8													
	Fard FNJ-N.						2	9	6	6	6			1			24.3													
	Deglet Noor N-9.					1	3	1	6	5	7	4	2	1			25.1													
	Ascherasi						3	3	4	9	9	1		1			24.9													
No. 9	Mosque.							5	5	9	5	5	1				25.1													
	Fard No. 4.				1		10	8	7	2	2						22.1													
	Fard FNJ-S					5	7	11	4	2	1						21.8													
	Canariensis No. 2	3	4	10	8	3	2										19.3													
	Deglet Noor N-9.						1	5	5	9	6	2	2				23.9													
	Ascherasi							6	9	10	2	3					24.6													
	Deglet Noor N-9.						1	4	5	13	4	2		1			24.8													
No. 10	Theory No. 20							1		4	11	6	4	4			25.6													
	Fard No. 4.								5	9	6	7	3				24.8													
	Fard FNJ-N.								3	5	8	11	3				25.2													
	Saidy No. 25.									1	4	6	9	7	2		26.9													
	Deglet Noor N-9.								3	6	12	7	2			1	26.0													
	Maktum No. 5.						4	5	11	7	2	1					23.0													
	Maktum No. 6.							5	6	6	7	5	1				24.1													
No. 13 (on Deglet Noor seedling No. 6).	Theory No. 20						1	4	5	8	8	3	1				24.0													
	Mosque.							1	8	4	8	9	1				23.7													
	Fard No. 4.						10	7	7	4	2						21.4													
	Canariensis No. 2.	6	7	10	6	1											18.6													
	Mosque (15)												4	2	7	2	27.4													
	Fard No. 4 (7)										1	3					25.7													
	Canariensis (20)										7	4		1			22.8													
No. 15 (on Khadrawy)	Mosque (20)				2	8	8	2									20.5													
	Fard No. 4 (20).				9	8	3										18.7													
	Mosque (8)					1	2	5									20.5													
	Fard No. 4 (20)	1	3	6	10												19.3													
	Maktum No. 5 (20)	1	1	12	5	1											19.2													
	Mosque.										3	5	9	5	6	1	26.4													
	Fard R.B No.1						1	1	8	9	5	5	1				24.2													
No. 18.	Mosque.							2		6	8	5	7	1		1	26.5													
	Fard A-21-2-32.						1	6	10	5	5	3					23.5													

TABLE 11.—*Breadths of date fruits and seeds in the 1926 experiments at Indio, Calif.*

[Measurements of 30 fruits were made except where a smaller number is indicated in parenthesis]

Experiment	Pollen used	Fruit measurements (millimeters) and frequencies														Seed measurements (millimeters) and frequencies									
		17	18	19	20	21	22	23	24	25	26	Mean	5	6	7	8	9	10	Mean						
No. 1.	Mosque.			4	13	11	2					20.4				21	8	1	8.3						
	Fard No. 4.		6	6	13	5						19.6			22	8		7.3							
	Canariensis No. 2.	3	12	7	5	2	1					18.8	1	15	14			6.4							
	Deglet Noor N-12.			9	12	6	3					20.1			16	12	2	7.5							
	Fard FNJ-S.		6	13	8	3						19.3			2	26	2	7.0							
	Fard FNJ-N.		1	8	11	7	3					20.1		3	20	5	2	7.2							
	Menakher No. 1.		2	4	11	9	4					20.3		3	11	15	3	7.7							
	Maktum No. 5.			7	11	8	4					20.3			6	20	4	7.9							
No. 2a.	Deglet Noor R-6.		2	10	10	7	1					19.8			4	20	6	8.1							
	Mosque.				6	13	11					21.2				13	17	8.6							
	Fard No. 4.			1	9	14	6					20.8			21	9		7.3							
	Canariensis No. 3.	1	5	4	10	8	2					19.8		16	13	1		6.5							
	Maktum No. 5.			2	11	7	9	1				20.9			9	19	2	7.8							
	Fard FNJ-S.		1	7	19	3						19.8			16	14		7.5							
	Fard FNJ-N.		1	12	10	7						20.8		3	21	6		7.1							
	Deglet Noor N-10.			8	12	8	2					20.1		1	25	4		7.1							
No. 2b.	Mosque.			3	12	10	5					20.6				15	15	8.5							
	Fard No. 4.			4	15	11						20.2		4	24	2		6.9							
	Deglet Noor N-18.			1		9	10	10				20.9		1	14	14	1	7.5							
	Mosque.		10	10	9	1						19.0			1	17	12	8.4							
	Fard No. 4.		2	14	12	2						18.5			1	11	18	7.6							
	Canariensis No. 3.	12	12	4	2							17.9	1	21	7	1		6.3							
	Government No. 1.		1	8	20	1						19.7			2	20	8	8.2							
	Mosque.		1	3	7	12	4	3				21.8			2	11	17	8.6							
No. 4.	Fard No. 4.		1	2	11	7	9					20.7		2	16	12		7.3							
	Canariensis No. 2.		2	7	14	6	1					19.9		12	18			6.6							
	Deglet Noor N-12.			2	15	9	4					20.5			26	3	1	7.2							
	Mosque.			3	13	9	3	2				20.6			2	21	7	8.2							
	Fard No. 4.		2	5	15	7	1					20.0		1	18	11		7.3							
	Saidy No. 13.		2	7	11	5	5					20.1			3	23	3	7.9							
	Deglet Noor N-18.		2	8	14	6						19.8			12	17	1	7.6							
	Mosque.		1	2	18	9						20.2			1	22	7	8.2							
No. 6.	Fard No. 4.		1	3	14	10	2					20.3			15	15		7.5							
	Deglet Noor R-1.			2	14	10	3	1				20.6			6	21	3	7.9							
	Deglet Noor R-6.		1	11	11	7						19.8			5	21	4	8.0							
	Fard No. 4.			2	9	14	5					21.1			25	4	1	7.2							
	Fard FNJ-S.			2	9	14	5					21.7			18	11	1	7.4							
	Canariensis No. 2.			4	4	3	4					20.5	1	4	9	1		6.7							
	(15).																								
	Maktum No. 5.		2	6	14	7	1					21.0			10	17	3	7.7							
No. 8.	Maktum No. 6.		3	3	16	4	4					21.1			12	18		7.6							
	Maktum No. 7.			10	9	9	2					21.1			4	24	2	7.9							
	Mosque.		1	5	10	8	6					21.4			5	18	7	8.1							
	Fard No. 4.		3	16	9	2						20.3		2	20	7	1	7.2							
	Menakher No. 1.			2	1	9	12	5	1			22.7			2	21	7	8.2							
	Theory No. 20.				5	11	13	1				22.3			4	21	4	8.1							
	Saidy No. 25.			3	9	14	2	1	1			21.7			8	18	3	7.9							
	Fard FNJ-N.			6	11	10	3					21.3			11	18	1	7.7							
No. 10.	Deglet Noor N-9.		1	3	12	8	6					21.5			7	20	3	7.9							
	Ascherasi.			3	10	16	1					21.5			5	23	2	7.9							
	Mosque.		1	7	14	6	2					20.0			1	17	12	8.4							
	Fard No. 4.		1	5	8	12	4					19.4		3	18	9		7.2							
	Fard FNJ-S.			4	16	9	1					19.2			19	11		7.4							
	Canariensis No. 2.	3	12	13	2							18.5		13	17			6.6							
	Deglet Noor N-9.	3	19	6	2							19.2			10	19	1	7.7							
	Ascherasi.			1	9	14	6					20.8			3	24	3	8.0							
No. 11.	Deglet Noor N-9.		1	9	11	8	1					21.0			8	22		7.7							
	Theory No. 20.		1	2	12	14	1					21.4			2	25	3	8.0							
	Fard No. 4.		1	5	14	10						21.1			24	6		7.2							
	Fard FNJ-N.			4	13	12	1					21.3			22	8		7.3							
	Saidy No. 25.			2	7	14	7					21.8			1	23	6	8.2							
	Deglet Noor N-9.			3	9	15	3					21.6			4	25	1	7.9							
	Maktum No. 5.		2	12	12	4						20.6			1	24	5	8.1							
	Maktum No. 6.	1	6	16	6	1						20.0			4	24	2	7.9							
No. 12.	Theory No. 20.		2	12	12	4						20.6			5	24	1	7.9							
	Mosque.			6	9	13	2					21.4			4	14	14	7.6							
	Fard No. 4.			4	10	12	4					20.5		4	24	2		6.9							
	Canariensis No. 2.	3	5	15	6	1						19.9		21	9			6.3							
	Mosque (15).			4	5	5	1					21.2				10	5	8.3							
	Fard No. 4 (7).		1	2		4						21.0			2	5		7.7							
	Canariensis No. 2.	1	2	13	3	1						20.1		1	13	6		7.3							
	(20).																								

TABLE 11.—*Breadths of date fruits and seeds in the 1926 experiments at Indio, Calif.—Continued*

Experiment	Pollen used	Fruit measurements (millimeters) and frequencies										Seed measurements (millimeters) and frequencies							
		17	18	19	20	21	22	23	24	25	26	Mean	5	6	7	8	9	10	Mean
No. 15 (on Khadrawy).	Mosque (20).....				2	2	11	4	1			22.0				9	11		8.6
	Fard No. 4 (20).....			2	5	9	4					20.8				4	16		7.8
No. 16 (on Maktum).	Mosque (8).....								6		2	24.5				1	6	1	8.0
	Fard No. 4 (20).....					1	7	11	1			22.6				10	10		7.5
	Maktum No. 5 (20).....						9	7	4			22.8				8	12		7.6
No. 17.....	Mosque.....			1	6	12	10	1				21.1				13	16	1	8.6
	Fard R.B. No. 1.....			8	13	6	3					20.1				16	14		7.5
No. 18.....	Mosque.....			1	9	12	7	1				20.9				2	14	14	8.4
	Fard A-21-2-32.....			6	15	7	2					20.2		2	17	11			7.3

In every experiment the pollen from Mosque produced larger fruit (fig. 6, D) and seed (fig. 5, B, and 7) than that from Fard No. 4. Since for these two pollens the breadths showed a tendency to vary in the same direction, the lengths afford the best basis for comparison. In all but one of the seven experiments on Deglet Noor in 1925 the mean difference of fruit lengths was more than three times its probable error,<sup>5</sup> the least significant difference in six of the experiments being  $1.6 \pm 0.16$  mm. in experiment 2 (100 measurements). The mean difference of seed lengths was significant in every one of the seven, the least being  $1.5 \pm 0.28$  mm. in experiment 6 (45 measurements). Considering each mean as a unit, the average mean difference<sup>6</sup> for these seven experiments was  $2.2 \pm 0.3$  mm. for the fruit and  $3.1 \pm 0.25$  mm. for the seed.

TABLE 12.—*Average of 10 measurements of fruits and seeds in the 1926 experiments in the Salt River Valley, Ariz.*

Experiments 1-7 were at the Tempe Date Garden, and experiments 8 and 9 were at the garden of the Arizona Orchards Company]

Experiment	Pollen used	Fruits		Seeds	
		Length	Breadth	Length	Breadth
No. 1.....	Mosque.....	42.1	21.1	25.1	8.5
	Fard.....	39.1	19.6	22.5	7.6
No. 2.....	Mosque.....	42.4	23.9	25.3	8.1
	Fard.....	39.0	21.0	22.2	7.4
No. 3.....	Mosque.....	42.4	21.2	24.9	8.4
	Fard.....	39.7	20.0	21.7	7.4
No. 4.....	Mosque.....	43.8	22.7	26.4	8.3
	Fard.....	40.6	20.0	22.5	7.1
	Fard FNJ-S.....	41.4	21.4	24.1	7.4
	Fard FNJ-N.....	41.5	21.3	22.8	7.3
No. 5.....	Mosque.....	42.9	21.6	24.6	8.8
	Fard.....	39.3	20.0	20.9	7.3
	Fard FNJ-S.....	40.8	20.9	21.4	7.4
	Fard FNJ-N.....	40.8	20.8	22.3	7.6
No. 6.....	Mosque.....	42.6	21.4	25.1	8.1
	Fard.....	40.0	20.4	22.5	7.3
	Fard FNJ-S.....	40.0	20.4	22.5	7.2
No. 7.....	Mosque.....	40.2	19.4	23.1	7.7
	Fard.....	38.8	19.3	21.9	7.9
No. 8.....	Mosque.....	43.2	23.6	26.4	9.4
	Fard.....	42.4	22.1	23.4	8.0
No. 9 (Iteema).....	Mosque.....	43.8	24.0	26.9	10.1
	Fard.....	41.0	22.5	23.5	8.6

<sup>5</sup> For the probable error of the mean the formula used is  $PE = 0.6745 \sqrt{\frac{\sum v^2}{n(n-1)}}$

<sup>6</sup> PEARSON, K. ON THE PROBABLE ERROR OF A COEFFICIENT OF MEAN SQUARE CONTINGENCY. *Biometrika* 10: 570-573. 1915.

All of the 1926 experiments on Deglet Noor at the Indio station showed a significant mean difference between the Mosque and the Fard No. 4 pollinations, the least for the fruit being  $1.3 \pm 0.21$  mm.

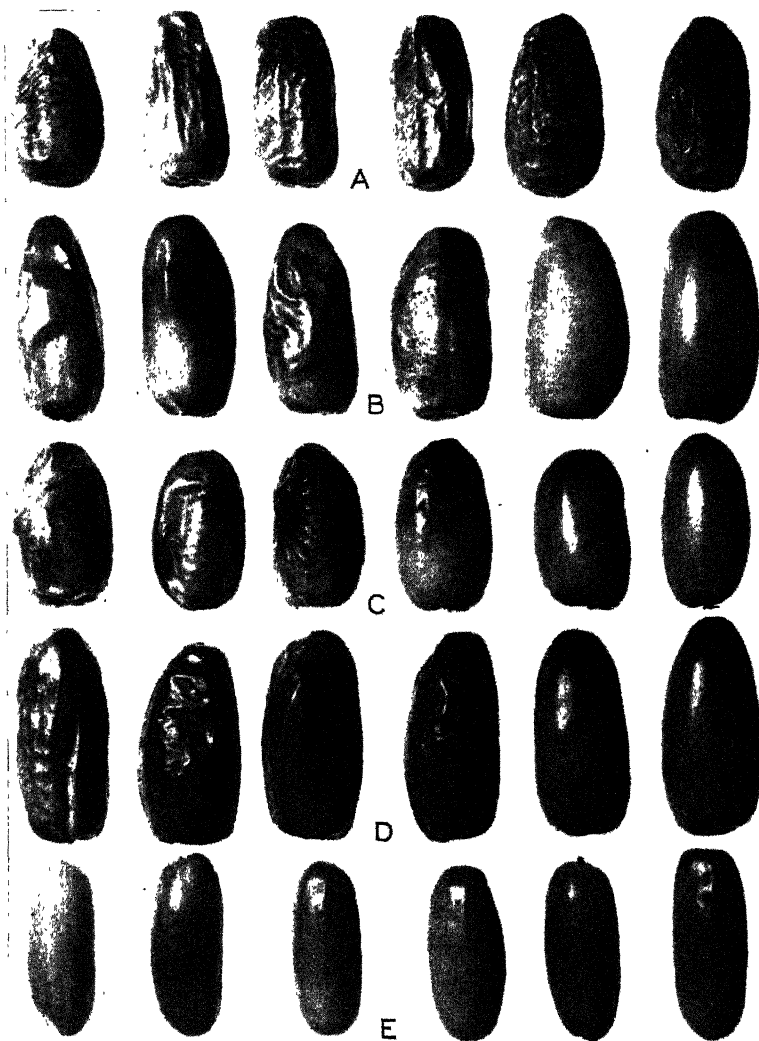


FIG. 6.—Typical dates of the Deglet Noor variety produced by different pollens on the same cluster in experiment 4 in 1925. The male palms represented are: A, Fard No. 4; B, Government No. 1; C, Canariensis No. 1; D, Mosque; and E, unpollinated dates. The dates from Fard No. 4 pollen were all full ripe on September 11, when the photograph was taken, but there were still immature dates on all the other strands.

and for the seed,  $1.8 \pm 0.21$  mm. The average mean for nine experiments was  $3.4 \pm 0.39$  mm. for the fruit and  $3.1 \pm 0.21$  mm. for the seed.

In the Salt River Valley experiments in 1926 ten measurements were made in each pollination. (Table 12.) These measurements were comparable to the larger number made at Indio, the average mean difference in length for eight experiments with Mosque and

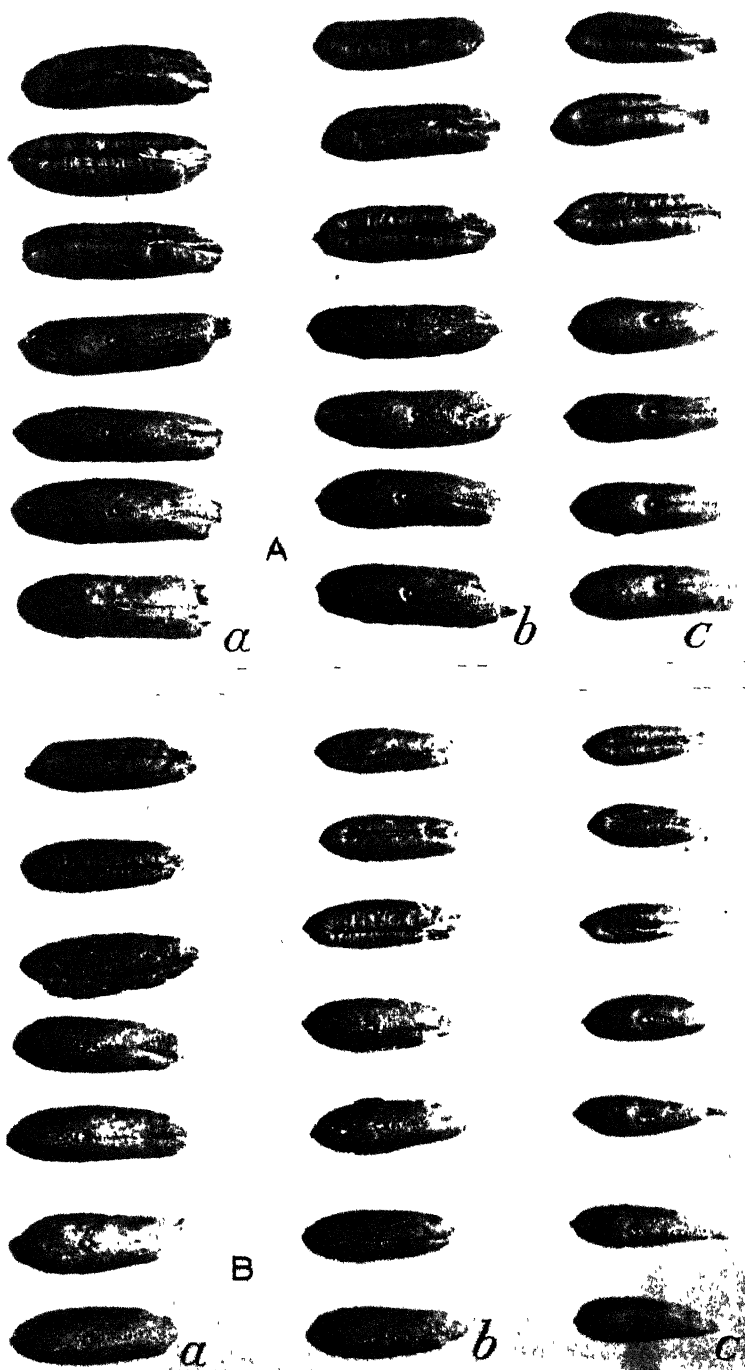


FIG. 7.—A.—Typical Rhars seeds produced on the same cluster by different pollens in experiment 14 in 1926. The male palms represented are: *a*, Mosque; *b*, Fard No. 4; and *c*, Canariensis No. 2. B.—Typical seeds from Deglet Noor seedling No. 6 produced on the same cluster by different pollens in experiment 13 in 1926. The male palms represented are: *a*, Mosque; *b*, Fard; and *c*, Canariensis No. 2.

Fard No. 4 on Deglet Noor being  $2.6 \pm 0.25$  mm. for the fruit and  $2.9 \pm 0.21$  mm. for the seed.

That this increase in size is accompanied by an increase in weight is indicated by the results of determinations in nine experiments with Deglet Noor at Indio in 1926. The mean weight of the seed was 102 gm. per 100 for the pollinations with Mosque and 68.3 gm. per 100 for the pollinations with Fard No. 4, a difference of  $33.7 \pm 1.85$  gm., or 49.3 per cent. For the fruit the mean dry weight of flesh only was 725.1 gm. per 100 for pollinations with Mosque and 623.2 gm. per 100 for the pollinations with Fard No. 4, a difference of  $101.9 \pm 21.54$  gm., or 16.4 per cent. In the weights, as in the measurements, it is apparent that the effect on the flesh is proportionately smaller and more variable than on the seed. By way of comparison, the seed in four experiments with pollen of *Phoenix canariensis* averaged 45.1 gm. per 100, while the dry weight of flesh only in three experiments averaged 602.8 gm. per 100.

It seems more than likely that these pollens will affect the fruit of other varieties in a similar manner. The results of two seasons' experiments with Deglet Noor seedling No. 6 and of a preliminary test with each of the varieties Rhars, Khadrawy, Maktum, and Iteema in 1926, included with the other tabulations, are entirely in harmony with the results obtained with the Deglet Noor variety.

#### DIFFERENCES IN QUALITY

So far no differences in the fruit as regards texture, flavor, etc., have been found to appear consistently in all of the experiments. This applies to all of the pollens tested. Through the courtesy of A. F. Sievers, of the Bureau of Plant Industry, United States Department of Agriculture, sugar analyses were made of the fruit in one experiment in 1925 with pollens from five male palms represented including Mosque, Fard No. 4, and Canariensis No. 1, and in two experiments in 1926, Mosque and Fard No. 4 being represented in both and Canariensis No. 2 in one. As all of the samples had been stored for some weeks before they were sent to the laboratory, the effect of storage may be questioned, but the results of the analyses did not indicate any significant differences in the sugar content. The likelihood that pollen has any direct effect on the sugar of the date would seem to be lessened by the fact that the sugar content of two samples of "unpollinated dates," one in 1925 and one in 1926, which finally ripened about three months after those which received pollen, varied less than 2 per cent from that of the nearest pollinated fruit.

Except in so far as the size and proportions of the seeds and fruits may be involved, the evidence in hand does not indicate any direct effect of pollen on the fruit which would properly come within the scope of the rather indefinite term "quality." But there is an indirect influence. In the Coachella Valley, Calif., it is a matter of common observation among date growers that Deglet Noor dates ripening very early in the extreme heat of late summer show a distinct tendency to be inferior in quality to the fruit which ripens later in the season when the weather is cooler. Perhaps the reverse may occur in other localities where climatic conditions are frequently unfavorable during the latter part of the ripening season. In either case, pollen by varying the time of ripening of the fruit might indirectly affect the quality

of a large proportion of the crop. Furthermore, it should be noted that differences in the time of ripening due to pollen may be at any season indirectly responsible for apparent differences in texture by causing one set of fruit to mature during a period of lower humidity than another. Hence, from a small number of experiments it might appear that one pollen was actually producing a softer date than another, whereas the results under other conditions with reverse fluctuations of relative humidity would be exactly the opposite.

In the seeds, the chief difference other than size produced by pollen was in the color. While the range was such that an individual seed could not have been identified with certainty on that basis, it was apparent in every experiment on Deglet Noor that the seeds resulting from pollinations with Mosque were lighter than those from pollinations with Fard No. 4, the former ranging from light drab (R. XLVI) to wood brown (R. XL), while the latter ranged from wood brown (R. XL) to snuff brown (R. XXIX). Seeds from pollen of *Phoenix canariensis* were even darker, verona brown (R. XXIX) being the prevailing shade. The peculiar tapering base produced by pollen of *P. canariensis* on the seeds of Deglet Noor fruit has already been mentioned. This tapering tendency in a lesser degree also occurred in the pollinations with *canariensis* on Rhars and Deglet Noor seedling No. 6.

#### OTHER MALE PALMS TESTED

If it were only by some fortunate accident that Mosque and Fard No. 4 proved to be so diverse and no other males could be shown to vary in such proportion, the immediate practical value of these experiments would be of somewhat less consequence, for the hope of the future would be largely dependent on breeding through a long period of years. However, it is now clear that there are *dactylifera* males equally as late as Mosque and others equally as early as Fard No. 4.

As mentioned above, from one to four preliminary tests were made at the Indio station with pollen from each of 20 other palms of *Phoenix dactylifera* and three of *P. canariensis*. Most of the *dactylifera* males ranged between Mosque and Fard No. 4, and, as might be expected from the nature of ripening there were many minor fluctuations. Yet at least five (Fard FNJ-S, Fard FNJ-N, Fard RB No. 1, Fard A-21-2-32, and Deglet Noor N-12, two of which are shown in Figure 3) appeared comparable to Fard No. 4 and three (Deglet Noor R-6, Deglet Noor N-9, and Huey, the last being designated by the name of the owner of the palm located near Bard, Calif.) to Mosque. It will be noted that of the male palms producing early-ripening fruits four of the five just mentioned are seedlings of the Fard variety. One of these (Fard FNJ-N) showed a tendency to ripen fruit even earlier than Fard No. 4. Whether any corresponding uniformity may occur among the seedling males of other varieties is for future tests to determine, but in these preliminary tests it did not occur among the several seedling males of the Deglet Noor variety, one of which was comparable to Fard No. 4 and two about on a par with Mosque.

The results of these preliminary tests will of course be subject to further verification, but from the consistent behavior of the pollens of Mosque and Fard No. 4 in every test during a period of two years



such verification may be expected to be largely a determination of the exact lateness or earliness of ripening produced. In other words, because of the range of experimental error a small number of tests may not indicate with certainty whether one pollen is a trifle later than Mosque or another a trifle earlier than Fard No. 4, yet there can be little doubt even from a few careful experiments that the one is definitely a late pollen and the other definitely an early one.

So far no exact correlation between size and earliness of fruit has been found. Yet this much is apparent, that among the male palms of *Phoenix dactylifera* no early pollens produced very large fruits or seeds and no late pollens produced very small fruits or seeds.

On the other hand, the smallest fruits and seeds produced in any of these tests were from the pollen of *Phoenix canariensis*. Pollens from two palms of this species, Nos. 1 and 2, ripened the fruit even later than the Mosque, while the third, No. 3, ripened the fruit somewhat earlier.

It seems very questionable whether the limits of variation have yet been reached. On the contrary, the diversity among the few already tested makes it more than likely that among the hundreds of males scattered wherever palms are grown there are many which will produce, and are producing, variations as great as or greater than those that appeared in any of these experiments.

#### SUMMARY

To test the direct effect of pollen on the fruit of the date palm, experiments were made at the United States Experiment Date Garden at Indio, Calif., in 1925 and 1926,<sup>7</sup> with additional tests in the Salt River Valley, Ariz., in 1926. Pollens from 25 staminate palms, 22 seedlings of *Phoenix dactylifera*, and 3 of *P. canariensis* were used on pistillate palms of the Deglet Noor variety. In some of the experiments successive inflorescences were pollinated, each with a different pollen. In most of them several pollens were applied, each to a different set of strands on the same inflorescence. Including two tests on a Deglet Noor seedling palm and one each on the Rhars, Khadrawy, Iteema, and Maktum varieties, two of the pollens which produced diverse effects were directly compared in 30 experiments. The resulting fruit and seed showed consistent differences according to the source of the pollen.

Significant differences were produced in the size and proportions of the seed.

Pollen from *Phoenix canariensis* affected the shape of the seed by producing a distinct, tapering base.

Variations in the size of the seed were accompanied by differences in the size of the fruit itself, though the latter were proportionately less than the former.

The most striking effect of pollen was a difference in the time of ripening of the fruit, which in some of the experiments was as much as 10 days in the early part of the season, with a tendency to increase in the latter part of the season.

<sup>7</sup> A still more extensive series of pollination experiments was conducted in 1927. The data, as far as they have been assembled and studied prior to the publication of this article, completely verify the results obtained in 1925 and 1926.

Xenia, known to occur in corn, wheat, and many other plants, may be the explanation of the direct effect of pollen on the seed, but it does not account for the influence on the fruit outside the embryo and endosperm. The data suggest an interrelation,<sup>8</sup> heretofore unrecognized, between the embryo and endosperm and the tissues outside.

These experiments indicate the importance of a further study of the pollination problems involved in date culture, but they are of immediate significance to date growers, emphasizing the need for careful selection of male palms. It is evident that pollen may be utilized as a factor in obtaining high-grade standardized fruit, and through its influence on the time of ripening it may be a means of adapting date culture to minor climatic variations.

<sup>8</sup> For the direct effect of pollen on the tissues of the mother plant outside the embryo and endosperm, Walter T. Swingle, under whose direction the experiments herein reported were initiated, has proposed the term "metaxenia," first in a report and discussion of the evidence at the meeting of the Southwestern Division of the American Association for the Advancement of Science at Phoenix, Ariz., Feb 15-18, 1926. Later a more complete discussion was presented in two papers read by Swingle before the International Congress of Plant Sciences at Ithaca, N. Y., August 16-23, 1926, under the following titles, abstracts of which appear in the proceedings of the congress: SWINGLE, W. T. HYPOTHETICAL EXPLANATION OF METAXENIA OR EFFECTS EXERTED BY THE MALE PARENT ON TISSUES OF THE MOTHER PLANT LYING OUTSIDE OF THE EMBRYO AND ENDOSPERM. [Unpublished manuscript.]

— and NIXON, R. W. THE EFFECTS EXERTED BY DIFFERENT POLLENS ON THE DEVELOPMENT OF THE FRUIT OF THE DATE PALM. [Unpublished manuscript.]

# DETERMINATION OF LENGTH OF TIME DURING WHICH THE FLOWERS OF THE DATE PALM REMAIN RECEPTIVE TO FERTILIZATION<sup>1</sup>

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## INTRODUCTION

Few experiments have been made and little published on the length of time after the opening of the flower spathe during which the pistillate flowers of the date palm (*Phoenix dactylifera*) remain receptive to fertilization by pollen from the staminate flowers. It has been believed that high percentages of fertilization may be obtained as long as two weeks after the opening of the flowers. Drummond<sup>2</sup> stated that pollinations up to the eleventh day after opening gave 98 per cent of fruit setting on the Deglet Noor variety, 50 per cent on Saidy, and about 25 per cent on Thoory. It is the recollection of the writer, who was identified with those experiments, that the control of chance pollination from outside sources was inadequate in the light of the results of the series of tests herein described.

In order to obtain more conclusive evidence, a series of experiments covering a period of four years (from 1923 to 1926) was carried on at the United States field station at Sacaton, Ariz., where date palms have been successfully grown and fruit produced for a number of years.

## METHODS

In the course of these experiments a method of protecting the female flowers from chance pollination was worked out. After the spathes had reached nearly their maximum size, but before they had begun to open, they were covered with transparent bags made of two layers of waxed paper of medium weight. The bags were carefully made by hand to fit the spathes rather closely and were air-tight except at the open end. When drawn down over the spathes they were tied tightly and protected with a thick layer of absorbent cotton to prevent the entrance of insects and to reduce the possibility of the entrance of wind-blown pollen. (Fig. 1.) In the experiments of 1924, 1925, and 1926, in addition to being bagged, the spathes were carefully washed with grain alcohol applied with a camel's hair brush. After this treatment the chance of wind-blown viable pollen remaining on the spathes was extremely remote. That the protection from extraneous pollination afforded by these measures was good has been evidenced by clusters which were allowed to remain covered for several weeks upon which no fertilized fruits whatever were found. After the bagging of the spathes they were kept under observation from day to day and the date of opening duly noted.

<sup>1</sup> Received for publication Aug. 29, 1927; issued March, 1928.

<sup>2</sup> DRUMMOND, B. PROPAGATION AND CULTURE OF THE DATE PALM. U. S. Dept. Agr. Farmers' Bul. 1016: 21-22. 1919.



FIG. 1.—Bagged spathes on seedling date palm, showing method of application of transparent paper bags

At intervals varying from 1 to 33 days after the opening of the spathes the bags were removed and pollination performed. Pollen collected from the same male palm was always used as far as possible each season, and in no case was more than one kind of pollen applied on the same tree. Tufts of sterile absorbent cotton heavily covered with the pollen were applied to each flower cluster and tied in place, the quantity of pollen thus brought into proximity to the flowers being considerably greater than if twigs of male blossoms had been used. The method of applying the pollen was the same in all cases.

## EXPERIMENTAL DATA

### EXPERIMENTS IN 1923

In the 1923 experiments 46 clusters on 8 palms were pollinated after periods of delay up to 11 days after the opening of the spathes. Three clusters were pollinated immediately after opening. Greater stress was laid on the periods of delay of 4 to 9 days, as it was believed that during this period the changes in the condition of the flowers might be most apparent. The results of these experiments are given in Table 1, in which the results from all the trees are combined, grouped according to the number of days that pollination was delayed, and averaged according to the number of clusters in each group. It will be noted from this table that after the first figures, which represent the results when pollen was applied as soon as the spathes opened, there is a gradual decrease in the percentage of fertilized flowers as the delay in application of pollen was lengthened, with the exception of the seventh day, when there was a slight increase which was not significant. The highest single percentage attained was 89.6 on a cluster pollinated immediately after opening, and the lowest was 7.2 on a cluster delayed 11 days, the limit of the delay in this group of experiments.<sup>3</sup>

TABLE 1.—*Percentage of date flowers fertilized in delayed-pollination experiments, Sacaton, Ariz., 1923*

Period of delay	Number of clusters pollinated	Percentage of flowers fertilized	Period of delay	Number of clusters pollinated	Percentage of flowers fertilized
0 day.....	3	73.6	7 days.....	5	59.2
2 days.....	1	89.0	8 days.....	7	46.0
3 days.....	1	83.0	9 days.....	6	35.3
4 days.....	6	70.0	10 days.....	4	26.3
5 days.....	6	65.7	11 days.....	2	23.2
6 days.....	5	54.3			

### EXPERIMENTS IN 1924

In the 1924 experiments the period of delay was extended to 14 days, and two clusters were pollinated in each period. Table 2 gives the results of these experiments. In this table it will be noted that there are some fluctuations in the figures, but the general trend of the average percentage of fertilized flowers is downward as the delay

<sup>3</sup> The results of these tests in 1923 were reported in the following circular: KING, C. J., and LEDING, A. R. AGRICULTURAL INVESTIGATIONS AT THE UNITED STATES FIELD STATION, SACATON, ARIZ., 1922, 1923, AND 1924, U. S. Dept. Agr. Circ. 372: 37-38. 1926.

increases. The percentage of flowers fertilized even in the periods of little or no delay is rather low, but it was true of this season that all of the pollinations made among the group of seedling palms of which those in the test were members were less effective than in most years.

TABLE 2.—Percentage of date flowers fertilized in delayed-pollination experiments, Sacaton, Ariz., 1924

Period of delay	Percentage of flowers fertilized	Period of delay	Percentage of flowers fertilized	Period of delay	Percentage of flowers fertilized
0 day.....	67.0	5 days.....	56.5	10 days.....	40.3
1 day.....	68.4	6 days.....	33.9	11 days.....	27.5
2 days.....	42.0	7 days.....	49.9	12 days.....	20.6
3 days.....	64.0	8 days.....	45.0	13 days.....	29.5
4 days.....	53.8	9 days.....	42.1	14 days.....	15.7

#### EXPERIMENTS IN 1925

In the 1925 experiments seven seedling palms were employed, but owing to the fact that on four some of the bags pulled loose from their bottom wrapping of cotton or were accidentally injured, the results from only three were taken into consideration. In these experiments the intervals between the delay periods were increased to five days instead of continuing with the one-day interval as in the two previous years. Table 3 gives the results of this group of experiments.

TABLE 3.—Percentage of date flowers fertilized in delayed-pollination experiments, Sacaton, Ariz., 1925

Palm No.	Period of delay (days)	Percentage of flowers fertilized
A-16.....	0	70.1
	4	67.7
	10	21.0
A-24.....	0	57.0
	5	48.3
	10	33.2
A-25.....	15	7.3
	1	78.8
	10	1.4
	15	.9

In this connection it is significant that in an experiment on the use of year-old pollen during the same season, where the paper bags sustained storm injury, the fewest fertilizations occurred on the cluster which opened 16 days before the bag was torn. This supports the theory that these fertilizations resulted from wind-blown fresh pollen and shows clearly that at 16 days after the opening of the spathe there were but few flowers still capable of being fertilized.

#### EXPERIMENTS IN 1926

In 1926, owing to the writer's transfer to another station, the investigations were necessarily of a very limited character. It was possible to work on only two clusters on a full-grown, well-isolated palm of the Deglet Noor variety. Pollen for this test was sent by mail from

the United States Experiment Date Garden at Indio, Calif., and came from two registered male palms. On receipt the pollen was shaken from the flowers and placed in glass jars by an assistant, the writer not coming into contact with it prior to the time it was applied to the flowers.

These experiments were conducted in a manner entirely different from those of previous years. Immediately after the rupture of the spathes they were opened and the blossoms bagged as quickly as possible. Instead, however, of inclosing the entire cluster in one bag, the threads were divided into groups of four, each group being covered with a narrow, double-thickness bag of oiled paper with a layer of cotton around the bottom of each bag, as in previous years. At the time these operations were carried on, no male palms were in bloom at any point in the vicinity, and the writer had been scrupulously careful not to come into contact with the pollen received by mail or any carried over from the year before. In addition to the bagged groups of threads, of which there were six on each cluster, one group of four was hand pollinated at once, but only after the other groups had been securely bagged. The pollen was applied by means of a small tuft of absorbent cotton, plentifully covered, and each blossom was touched in the process. The tuft of cotton was afterwards tied among the threads, which were then left uncovered. The applications of pollen were spaced at 4-day intervals up to 20 and 16 days on the two clusters respectively, after which longer intervals were allowed. After each group was pollinated, the bag was left off and no further attention given it.<sup>4</sup>

The results obtained in these experiments are given in Table 4. It will be noted that the percentage of fertilized flowers even on the groups of immediate pollination and those of the shorter delays was rather low. The reason for these low percentages is probably closely connected with the weather conditions at the time. During the earlier part of the experiments the weather was far from ideal for date pollination. Considerable cloudy weather with frequent showers occurred during the first 10 days, and this was undoubtedly the principal factor in the low percentages of fertilization. This is clearly indicated in the pollination which was made on March 9 on cluster A, 8 days after the opening of the spathe. On this day and the one following the weather was cloudy most of the time, with frequent showers of rain. At the time of the next pollination, March 13, more favorable conditions prevailed, and though the delay was 4 days greater, over 9 per cent more fertilizations occurred. Similarly, on cluster B, on the same date (March 9), a slightly lower percentage of fertilization occurred than on March 13 and 17, 4 and 8 days later, respectively, when weather conditions were more favorable.

That the weather is a factor influencing successful date pollination is of course well known among date growers, and these instances are cited here merely to indicate the probable cause of the low percentages which occurred at the beginning of the experiment when higher ones might have been expected.

<sup>4</sup> The writer is indebted to Harold Fulton, of the Office of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, for assistance in handling pollen and making pollinations in his absence on Mar. 9.

TABLE 4.—Percentage of date flowers fertilized in delayed-pollination experiment, Sacaton, Ariz., 1926

Dat pollinated	Period of delay (days)	Number fertilized	Number unfertilized	Percentage of flowers fertilized
Cluster A:				
Mar. 1.....	0	126	156	44.68
Mar. 5.....	4	123	116	51.46
Mar. 9.....	8	111	189	37.00
Mar. 13.....	12	134	157	46.05
Mar. 17.....	16	65	212	23.46
Mar. 21.....	20	71	223	24.15
Mar. 30.....	29	24	235	9.27
Cluster B:				
Mar. 5.....	0	123	207	37.27
Mar. 9.....	4	142	203	41.16
Mar. 13.....	8	132	188	41.25
Mar. 17.....	12	146	198	42.44
Mar. 21.....	16	136	207	39.65
Mar. 30.....	25	66	271	19.58
Apr. 7.....	33	35	269	11.51

## DISCUSSION

While there has been some fluctuation in the percentages of fertilized flowers in these experiments, and a high percentage has seldom been obtained even where pollination was prompt, it must be borne in mind that during the first three years the work was carried on in a planting of about 200 seedling palms, no two of which were alike in character or behavior. A certain part of the variation which is found in the figures is no doubt due to the physiological differences in the plants and would not have been so evident had the work been possible on a number of palms of the same variety. There is some evidence, not as yet subjected to rigid experimental test, that in the case of a few date varieties similar physiological differences exist, limiting the time of delayed pollination. As has been explained, weather conditions had considerable bearing on the results of the 1926 experiment.

## CONCLUSIONS

On the whole the results obtained in these experiments clearly indicate that the longer pollination is delayed after the opening of the spathe the fewer fertilizations will be effected, and that when the object to be desired is a heavy setting of fruit it is unwise to delay pollination more than a few days. That high percentages of fertilized flowers may be obtained after pollen has been withheld for periods of 10 days or more is definitely controverted by the results of the work herein reported.



# DISPERSAL OF THE COTTON-BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOH.<sup>1</sup>

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## INTRODUCTION

Many flying insects have definite periods during the year when they leave their breeding grounds and disperse to other places by means of flight. The cotton-boll weevil is no exception. Although rather sluggish at certain times of the year and rarely using its wings, this species during the midsummer months becomes restless and flies readily from field to field in what is popularly termed the "migration." Prior to this time the adult, when disturbed, folds its legs beneath its body and often drops from the plant, feigning death or "sulling." These insects act altogether differently during the flight period. They are alert, and upon the slightest disturbance usually take to flight.

This phase of weevil behavior was studied during 1924 and 1925 as a part of an investigation of the biology of this pest at Florence, S. C. This paper deals primarily with the above-mentioned general summer-flight period of the species, and not with the less extensive dispersal habit of the female weevil after she becomes sexually mature.

## HISTORICAL

The fact that the boll weevil has a summer dispersal period during which it flies considerable distances was first discovered by H. A. Morgan in 1904. Hunter and Hinds (5)<sup>3</sup> discussed this fact, stating that on the Louisiana line, where this species was advancing, several distinct movements were observed. They noted that the flights began soon after the period of maximum infestation was reached. Newell (8) stated that this was induced or accelerated by an insufficiency of uninfested squares. He observed that the dispersal was not a daily continuous movement but that marked distinct migrations occurred several days apart. Hinds and Yothers (3) noted that the most extensive flights came between August 15 and September 20, when the cotton plant had practically ceased to form squares and there was no food available. Hunter (4) wrote that this movement took place frequently when fields were only slightly infested, stating that "the insect has a well-developed instinct for extending its range into new territory." He also observed that it did not fly in any particular direction unless governed by the wind and that "if there is no wind or only a light one, a weevil is as likely to fly in one direction as in another." Hunter and Pierce (6) divided natural dissemination as follows: (a) Spring search for cotton, quoting A. C. Morgan's findings at Victoria, Tex., that the fed male could fly as far as 775

<sup>1</sup> Received for publication Apr. 13, 1927; issued March, 1928.

<sup>2</sup> The writers are indebted to E. C. Bolt and A. M. Woodside, temporary assistants, for carrying out various details of the investigation here reported.

<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 149.

yards, the fed female 350, the average being 63.5, as compared with 66.6 yards for unfed weevils; (b) spring spread within the fields, when the insect travels at an average rate of 0.35 foot per day; (c) summer flights; (d) fall dispersion, this being due to a scarcity of food and breeding places because of maximum infestation, and also to an instinct to invade new territory; and (e) hibernation flight, which began before a lowering of the temperature. Hinds (1) found that in the spring for more than six weeks the adults had not moved more than 50 yards from the point at which they started and concluded that the field-to-field movement did not take place so long as uninfested squares continued abundant. Finally, Hinds and Bradley (2) stated that this migration was coincident with the occurrence of considerable numbers of weevils in the blooms.<sup>4</sup>

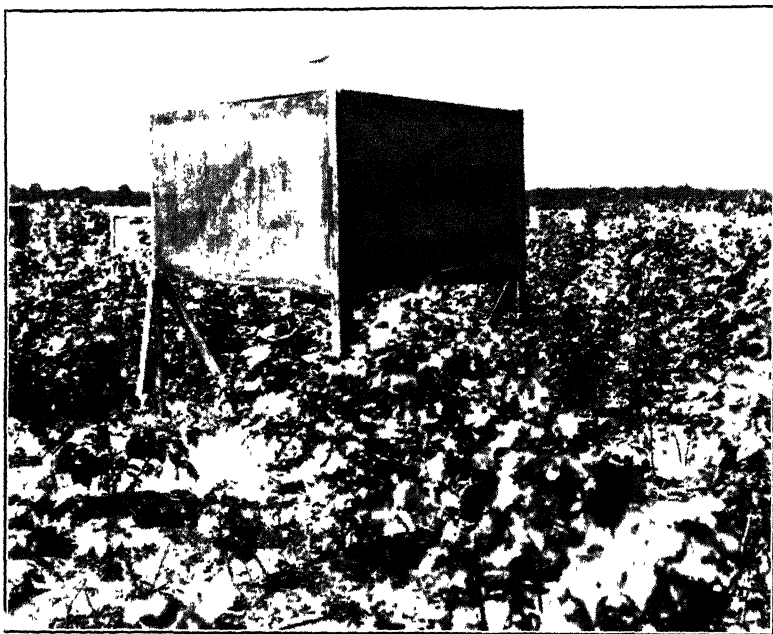


FIG. 1.—Screen trap used in studies of boll-weevil dispersal

#### METHODS

In order to determine when weevils were actually flying about, a number of screen traps were set up in or near cotton fields. (Fig. 1.) Each trap was made by tacking a piece of ordinary window screening 10 by 3 feet to three stout pieces of wood about 8 feet long, one at each end and one in the middle of the wire. Three holes were dug in the ground in a triangular arrangement to receive the supports of the screen, so that when the latter was in place the two wings formed a right angle and the lower margin was about 3 feet above the surface of the ground. The whole structure was securely braced so that the wire was taut. The screening was then thickly coated with a commercial sticky tree-banding preparation. This in turn was bordered

<sup>4</sup> Since this manuscript was written, an important paper on early summer dispersion of the boll weevil has been published by Dwight Isely (?).

with a heavy creosote mixture commonly used in banding trees as a protection against gipsy-moth caterpillars. The latter precaution was found to be necessary to prevent weevils from escaping on hot days when the commercial preparation often failed to hold them. These traps were examined twice daily and all weevils removed and counted.

#### DISPERSAL IN 1924

The first trial screen trap was under observation in 1924. This was set up July 29 in the center of a cotton field, and the first weevil was captured on it August 7. From this date up to November 14

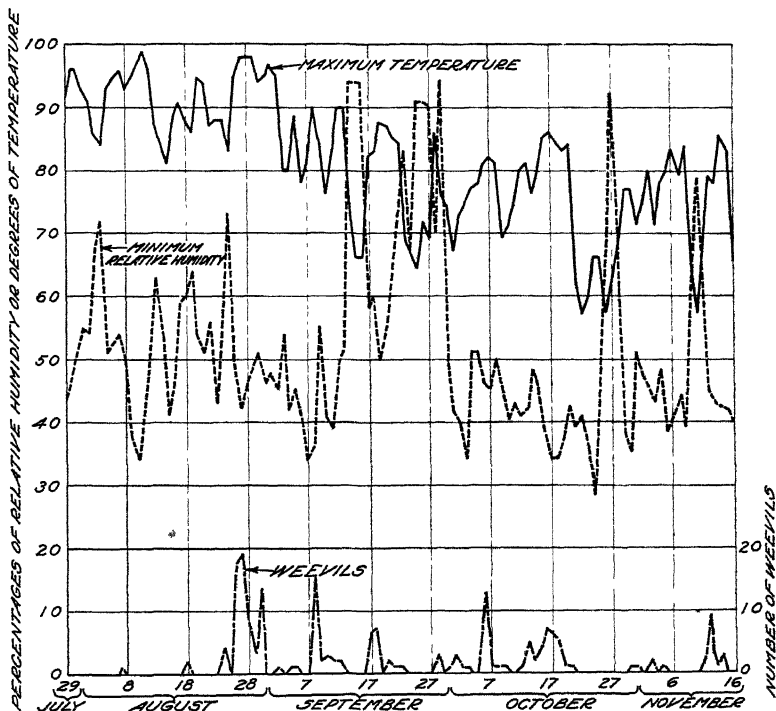


FIG. 2.—Number of boll weevils caught on screen trap, and records of minimum relative humidity and maximum temperature; Florence, S. C., 1924

the insects were in flight at various periods. These periods were more or less definite and came on the following dates: August 26 to 30, September 8 to 18, October 6, October 13 to 18, and November 11 to 14. (Fig. 2.) The weevils were in flight in greatest number during the first period and were less numerous in each successive flight. There was considerable range in the maximum temperatures when dispersal took place, but the lowest was 67° F. More were caught when the daily maximum temperatures ranged between 81° and 96°. On comparing the flight of this species with climatological data, it was evident that other factors modified the influence of temperature and humidity on flight. A careful field-to-field survey conducted over a wide territory when dispersal first began showed that cotton had practically stopped fruiting in most fields and bolls

were opening generally. Lack of food and unsatisfactory breeding places in this case seemed to be the cause of the movement. Rains continuing through the day checked flying, which in one case was resumed as soon as the weather became favorable.

#### PROPORTION OF SEXES CAUGHT AND TIME OF FLIGHT

A total of 199 weevils were captured on this screen, the sex of most of which was determined. There were 110 males and 88 females. From the time dispersal first began up to September 17 most of the adults were caught on the screen between 9 a. m. and noon, comparatively few being in flight during the afternoon. From this time on the proportion was reversed, and the majority were trapped during the afternoon.

#### DISPERSAL IN 1925

In 1925 six screens were set up in cotton fields and two at some distance from cotton. It was found that the time of dispersal in all the five unpoisoned fields was different from that in the poisoned field, and that the time of dispersal outside of the fields was different from that in the fields. The data have therefore been assembled according to the location of the screens and are discussed in the following paragraphs accordingly.

In all fields where a screen trap was located, except one, there were from one to three plots staked off, each composed of 100 plants. Weekly examinations were made to determine the number of weevils in each plot, as well as the number of squares, blooms, and bolls on the plants. The infestation of all fields was also noted and in addition several hundred infested forms were picked up and dissected for weevil stages, parasites, etc. These examinations were begun May 21 and continued until November 10.

#### WEEVIL FLIGHTS IN HEAVILY INFESTED FIELDS

Screens were set up in five fields which were not poisoned at any time during 1925. The first was surrounded by very favorable hibernation quarters. Early counts here showed that from May 21 to July 1 the overwintered weevils averaged 6.14 per 100 plants. They practically destroyed all cotton in this field. (Fig. 3.) The first adults were found on the screen trap July 17, a day after it was set up, but it is very probable that some were flying about before that date. The last one was captured on the screen October 15, but the greatest numbers were caught prior to September 16. There were two rather definite periods during which large numbers were in flight, namely, July 20 to 28 and August 7 to 17.

The second field was about as heavily infested, averaging 5.2 weevils per 100 plants. The soil was more fertile, however, and the cotton never entirely ceased square production. While the insects damaged this crop severely, the injury was not so severe as in the first field. The screen trap was set up July 17 and weevils were found on it the next day. The last one was caught on this trap October 21. The greatest number were taken between July 18 and 28, which was about the same time as recorded for the first heavy flight movement in the first field. However, unlike the above, there was no definite heavy flight period after this one.

The third field was located near favorable winter quarters for the weevils, but had been in corn the year before, and this may account for the smaller initial infestation by these insects, the count averaging 2.4 per 100 plants. The first weevils were caught on this trap July 17, the day after it was set up in the field, and the last one October 19. The largest number were in flight between July 18 and August 21.

The fourth field had fewer early overwintered weevils, the counts averaging 2 per 100 plants. The screen in this field was set up July

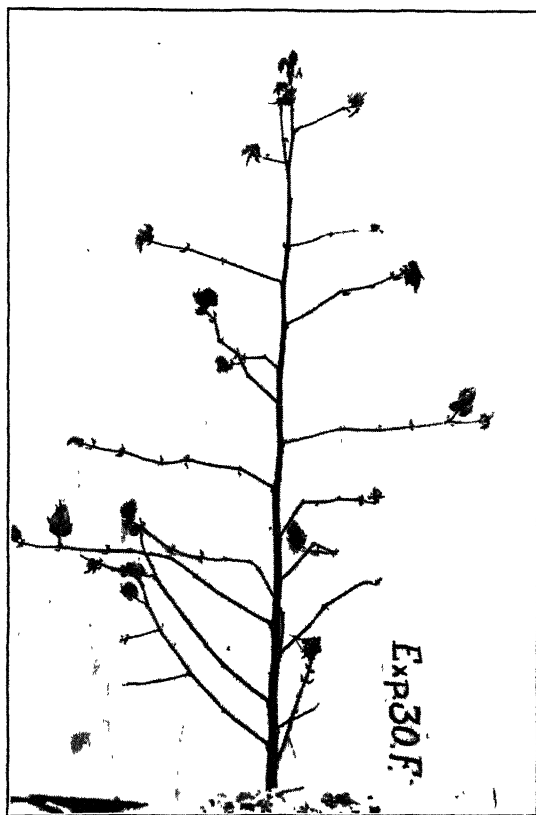


FIG. 3.—Appearance of typical cotton plant (after foliage had been stripped off by hand) in field where screen B was located, photographed August 14

23, and the next day weevils were taken from it. Weevils had undoubtedly been flying for some time before this date, as the largest daily catch was recorded July 24. The greatest number were caught on this trap from this date to August 1, and the last one was taken October 19.

The fifth field had the smallest number of overwintered weevils, the counts averaging 1.5 per 100 plants. The trap was set up July 23, and weevils were caught the next day in larger numbers than in any following 24-hour period. The largest number were in flight between July 24 and August 18, and there was also a smaller flight

from August 31 to September 3. None were found on the trap after October 2. A good crop of cotton was picked from the two last-mentioned fields, chiefly owing to the fact that the bottom crop was set before the infestation caused by migrating weevils became high. (Figs. 4 and 5.)

The summarized data on flight activities, as indicated by the screen traps in all of the five unpoisoned fields, show that weevils were taken from these the day after they were set up. Thus weevils

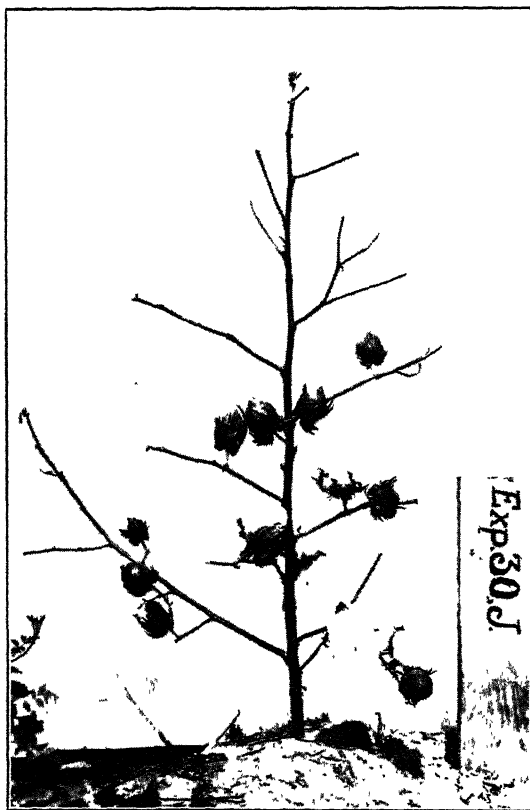


FIG. 4.—Appearance of typical cotton plant (after foliage had been stripped off by hand) in field where screen E was located, photographed August 14

were probably flying about to a certain extent before July 17. The heaviest flights came between July 17 and August 17, the peak being reached July 24. (Fig. 6.)

#### WEEVIL FLIGHTS IN POISONED, LIGHTLY INFESTED FIELD

The weevil dispersal in one partly poisoned field came later than that in the other fields under observation. The flight movement started July 28 and extended through October 21. The period of greatest flight came between August 7 and September 7, the peak being reached September 5. During this time high temperatures prevailed, the minimum humidity ranging somewhat lower than during

the few weeks prior to the flight period. No weevil counts were made in this field. The fruiting of the plants was approximately the same as on all other cotton in this vicinity.

Since half of the cotton in this field was poisoned, and the initial infestation by overwintered weevils was very small, it is believed that most of the weevils caught on the migration screen came in from other fields more heavily infested. This belief is supported by the weekly square counts, which showed the infestation to be as follows: July 1, 1.6 per cent; July 7, 4.4; July 13, 4.2; July 17, 5.0; July 22, 3.6; July 27, 3.0; and August 1, 13.6.

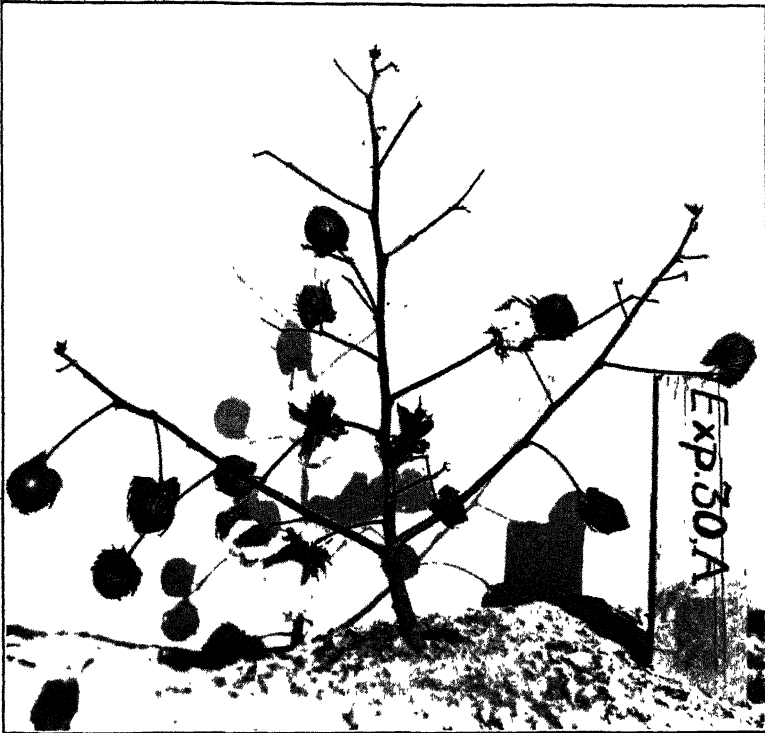


FIG. 5.—Appearance of typical cotton plant (after foliage had been stripped off by hand) in field where screen G was located, photographed August 14

Infestation had begun to increase by August 1, which was before the heaviest weevil movement recorded in this field. At this time square shedding had also begun, a fact which may partly explain the increase at this time. Coincident with the trapping of large numbers of weevils on this screen, the infestation of this field became practically complete.

#### WEEVIL FLIGHTS BETWEEN FIELDS

One screen was set up in an uncultivated field at a considerable distance from cotton and another in the center of a cowpea field bordered on three sides by cotton. Fewer weevils were caught by these traps than by those located within cotton fields. The first adult was taken July 28 and the last one October 16. A total of 2

were trapped in July, 23 in August, 9 in September, and 2 in October. The records pertaining to these screens show that most of the weevil flights between fields came during August, and more particularly between August 10 and September 2.

#### PROPORTION OF SEXES CAUGHT AND TIME OF FLIGHT

A total of 912 weevils were caught on the screens, 897 of which were classified as to sex (Table 1), 541 being males and 356 females. Four hundred and ninety were caught in the morning and 367 in the afternoon. When the other 55 were in flight was not determined.

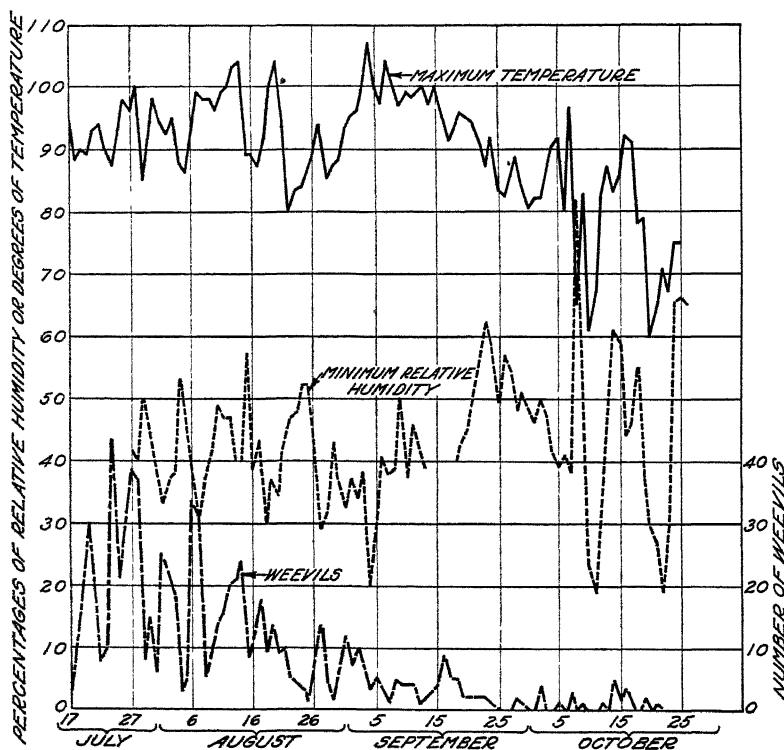


FIG. 6.—Number of boll weevils caught on six screen traps, and records of minimum relative humidity and maximum temperature; Florence, S. C., 1925

TABLE 1.—Number of male and female weevils captured, and number captured in the forenoon and afternoon, on migration screens, Florence, S. C., 1925

Screen	Male	Female	Sex undetermined	Total	A. m.	P. m.	Time undetermined	Total
A.....	126	92	4	222	125	93	4	222
B.....	83	47	3	133	84	39	10	133
C.....	72	52	.....	124	63	51	10	124
D.....	144	82	2	228	108	98	27	228
E.....	37	31	2	70	35	33	2	70
F.....	6	7	2	15	5	8	2	15
G.....	62	35	2	99	59	40	.....	99
H.....	11	10	.....	21	11	10	.....	21
Total.....	541	356	15	912	490	367	55	912



## TRAP-CROP RECORDS, 1925

In 1925 a plot of ground, one-tenth of an acre in extent, was planted to cotton for the purpose of determining when and how long weevils came into cotton fields from hibernation. This planting was examined daily and all weevils were removed. According to the cage records, these insects were through emerging from hibernation by July 1. To be sure that any individuals which might have been overlooked were eliminated, the plot was thoroughly dusted with calcium arsenate. Daily examinations were continued for a week after the dusting but only one weevil was found and killed. The cotton was then free from the insects. Infestation counts were made weekly by examining from 600 to 800 squares at various intervals throughout the plot. (Table 2.) The first signs of weevil damage were noted July 13, when 17 punctured squares were found in one spot, making a total infestation of 2 per cent. On July 16 this dropped to 0.61 per cent, only four punctured squares being found. On July 24 it had increased to 2.83, and from this date it increased rapidly up to 62.66 per cent on August 5. Since all overwintered weevils had been removed from this plot, and no opportunity was given them for reproduction, it is evident that the infestation was produced by migratory weevils coming in from neighboring cotton. These had to fly either through an extensive pine grove, or across an 8-acre tobacco field. The trap-crop records, therefore, check closely with the migration-screen records.

TABLE 2.—Records of infestation and plant fruiting in trap crop; Florence, S. C., 1925

	July 9	July 13	July 16	July 24	July 29	Aug. 1	Aug. 5
Percentage of infestation.....	0	2.00	0.61	2.83	5.70	20.0	62.66
Average number of squares per plant.....	13.00	18.00	23.33	18.70	8.30	7.0+	2.90
Average number of blooms per plant.....	.40	.91	1.00+	2.00+	.93	1.0	.44
Average number of bolls per plant.....	.72	1.40	5.00—	11.00	12.30	16.0	14.00

## RELATION OF DISPERSAL TO ENVIRONMENTAL FACTORS, 1925

## RELATION OF DISPERSAL TO MAXIMUM TEMPERATURE

During the period when most of the weevils were in flight, daily maximum temperatures varied from 85° to 104° F., the range usually being between 90° and 100°. (Fig. 6.) There was also a well-defined period, from August 28 to September 26, during which the daily temperature maxima were unusually high. On September 4 a peak of 107° was reached. This was followed by a rather definite decline. During this period there was a gradual decrease in the number of weevils caught on the screens. In general, more of these insects were in flight when the maximum was 80° or above, the most favorable temperatures being between 90° and 100°. The coefficient of correlation between maximum temperature and weevil flight was  $0.436 \pm 0.088836$ , which indicates a decided correlation. Since there were days when temperature conditions were favorable and yet no weevils were caught on the screens, this factor was of secondary importance.

## RELATION OF DISPERSAL TO MINIMUM RELATIVE HUMIDITY

During the time when most weevils were flying, minimum relative humidity readings showed daily fluctuations from 29 to 57 per cent, but there were no definite periods when there were any marked changes. (Fig. 6.) After this time, when the number of weevils caught on the screens began to drop off, the minimum relative humidity records were somewhat lower for a time. No extensive fluctuations occurred, however, until after October 7. The coefficient of correlation between these two factors was such as to indicate that relative humidity was a slight and unimportant factor in weevil flight activities.

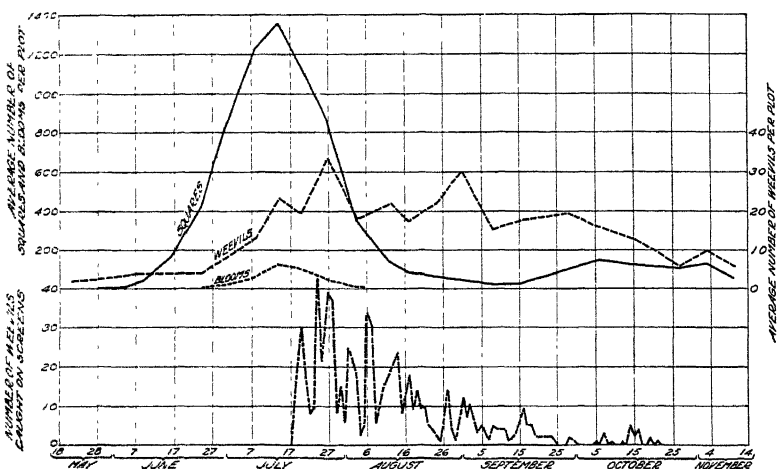


FIG. 7.—Number of boll weevils caught on six screen traps, and records of average bloom and square counts and average weevil population; Florence, S. C., 1925

## RELATION OF DISPERSAL TO RAINFALL

Rainfall was scattered and far below the average for this section. Also it usually came before or after the daily flight periods of the weevil. No correlation was noted between weevil flight and rainfall.

## RELATION OF DISPERSAL TO SQUARE PRODUCTION

In 1925 the earliest plants began to fruit on or about May 28. From this time on there was a very rapid increase in the total number of squares produced up to July 14,<sup>5</sup> when the peak was reached. Following this, there was an equally rapid decrease until August 12, when there were 1.45 squares per plant. From this date until September 8 the number of squares continued to decrease. Then there was a gradual increase up to October 6, but even at this date there was an average of but slightly more than one to a plant. After October 6 the number of squares decreased gradually to the end of the growing season, with a slight temporary increase on November 3.

In comparing the weevil flights with square production (fig. 7) it is seen that the flights began directly after the peak of production had passed. The greatest number of weevils were in flight when

<sup>5</sup> Average date for all fields.

the available square supply was diminishing rapidly. Then, as the number of squares continued to decrease, the number of weevils flying in the cotton fields decreased, but, as previously noted, the screens outside of cotton fields showed that a considerable interfield movement was taking place. When squares began to develop again there was no corresponding increase in flight activities, but rather a continued decrease. Therefore there was no high degree of correlation between weevil flights and square production in 1925.

#### RELATION OF DISPERSAL TO WEEVIL POPULATION

From May 21, when counts were started, to June 30 there were relatively few weevils in cotton, but a slight increase occurred from week to week as a result of their continued emergence from hibernation. From June 30 to July 14 there was a rapid increase in the weevil population because of the emergence of the first generation. On July 20 a decrease was noted, but on July 27 there was a considerable increase. On this date there were more weevils in some of the count plots than on any other date in 1925. Fewer were present in the fields from July 27 to August 31, when a second high peak was reached. From this date to the close of the season there were not nearly so many of the insects present in the fields.

Comparing the two curves representing weevil dispersal and population (fig. 7), it is seen that the flights began shortly after the first high point in the weevil count had been reached because of the emergence of the first generation. The heaviest flights were recorded about the time the greatest number were present in the field, when the second generation was issuing. When the second highest point in population was reached August 31, there was no corresponding increase in numbers flying but rather a decrease. Thus there seemed to be no marked correlation between these two factors.

#### RELATION OF DISPERSAL TO AVAILABLE FOOD SUPPLY AND PLACES FOR BREEDING

While there was no high degree of correlation between time of weevil dispersal and weevil population, or between weevil dispersal and square production, there was a direct relationship among the three taken together. In other words, the percentage of infestation of cotton forms had a direct bearing on weevil flight activities. When the flights began the available square supply was decreasing and the weevil population was increasing. Infestation counts taken July 24 and 25 showed that in four of the five unpoisoned fields the weevils had punctured most of the forms. In the five fields the infestation ranged from 31.6 to 88 per cent, the average being 62.9 per cent. This shows that the weevils were flying about when unpunctured squares began to be scarce. On the other hand, later on in all fields there was a considerable period during which the plants had few or no squares and young bolls developed, and weevils were present in large numbers. Under these apparently favorable conditions for dispersal, only a few weevils were captured on the screens situated within the fields. The two traps located outside of cotton fields, however, showed a considerable interfield movement at this time. The small number of weevils flying when cotton began to fruit again is explained by the fact that for a certain period of time after the top growth of cotton is resumed, the weevil infestation decreases. Late-developed

weevils also feed on the squares, apparently in preparation for hibernation, and there is not so much oviposition by the females. There is thus less stimulation for dispersal than is found earlier. From the foregoing facts it is evident that the dispersal of the boll weevil was first manifested by short flights from row to row, or from one part of a field to another, and that this was induced by a lack of uninfested squares and young bolls. The screens located in cotton fields detected this phase of the dispersal. Later, when the infestation was nearly complete, the flights were extended so that the weevils were dispersing from field to field, as was shown by the traps situated outside of cotton fields. Some of these insects also flew to neighboring cotton fields before the general movement took place, as was proved by the trap-crop records. There was, therefore, a direct correlation between weevil flights and lack of food and breeding places.

#### RELATION OF DISPERSAL TO DIRECTION OF WIND

A record was kept of the number of weevils caught on the four sides of the screen and also of the direction of the wind, both morning and afternoon. These insects were not blown by the wind, nor did they drift with it, for only 33.01 per cent were caught on the windward side of the screen. (Table 3.) Nor did they fly against the wind so far as could be determined. Apparently, weevils were not influenced to any appreciable extent by moderate winds.

TABLE 3.—*Effect of direction of wind on dispersal of boll weevil; Florence, S. C., 1925*

Direction of wind	Number of weevils caught on sides of screen						Number of weevils drifted	Percentage of drift
	North	South	East	West	Undetermined	Total		
North.....	35	31	41	27	4	138	35	26.12
Northeast.....	16	24	16	29	2	87	32	37.65
Northwest.....	8	8	6	5	1	28	13	48.15
South.....	46	43	50	58	14	211	43	21.83
Southeast.....	26	28	31	18	1	104	59	57.28
Southwest.....	38	34	29	40	56	197	74	52.48
East.....	6	6	9	14	1	36	9	25.71
West.....	7	4	9	9	1	30	9	31.03
Calm.....	20	9	26	15	1	71		
Undetermined.....		3	4	2		9		
Total.....	262	190	221	217	81	911	274	33.01

#### RELATION OF DISPERSAL TO EMERGENCE OF THE DIFFERENT GENERATIONS

The dates of emergence of the different generations of weevils in 1924 were as follows: First, June 26 to September 30; second, July 24 to October 29; third, August 17 to November 14; and fourth, September 12 to October 31. At the time dispersal began in large numbers, namely, on August 26, there were weevils of the first, second, and third generations in the field. (Fig. 8.)

In 1925 the dates of emergence of the four generations of weevils were as follows: First, July 1 to August 17; second, July 23 to October 4; third, August 20 to September 23; and fourth, October 19 to November 28. Dispersal began July 17 and was at its height between July 24 and August 7. The dispersal flight of the weevil

in 1925 also showed little relationship to the emergence of the different generations. (Fig. 9.)

From the time the first summer-generation weevils begin to issue from the infested squares, which is usually late in June or early in July in this section, until the first killing frost in October or November, there is a steady daily emergence of newly transformed weevils, either from infested squares or bolls. The number varies according to the fruiting condition of the cotton plant or according to climatic conditions, and there is a so-called "peak" to the emergence of any one generation. If there were a direct relationship between migration and the life history of the weevil, there would be a more or less

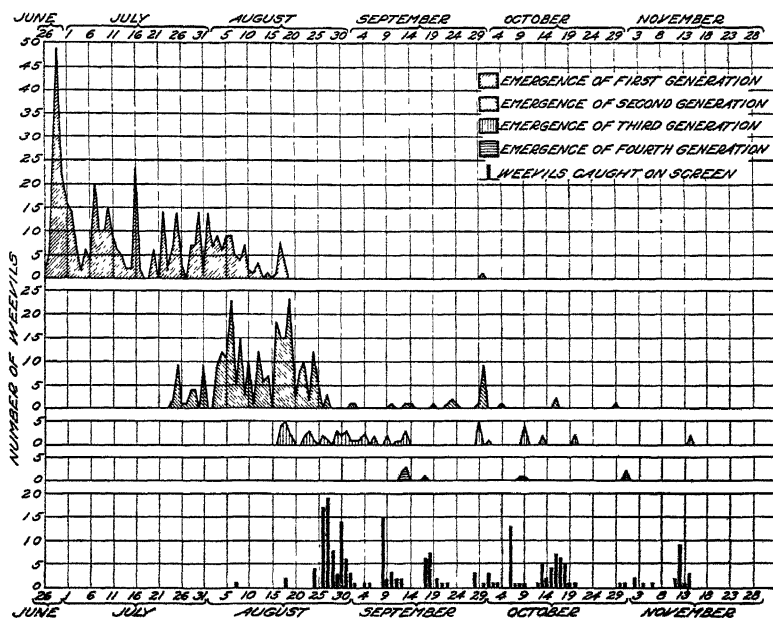


FIG. 8.—Emergence of different generations of boll weevils in 1924 and number of weevils caught on screen trap

continuous daily movement of weevils, fluctuating, of course, with weather conditions, increasing as the weevil population increased, or when a peak of emergence was reached. This, however, did not occur.

#### RELATION OF DISPERSAL TO DIRECTION OF FLIGHT

It was noticed that on all screens where considerable numbers of weevils were caught, more were found entangled on one side of the trap than on the others. Sometimes this varied throughout the season. For example, on the screen in the poisoned field more were collected from the east side up to August 14. After this time more came in from the north, until by August 20 the accumulated totals collected on this side exceeded those from the east. On September 5 the flight was from the east. On the two days following about the same number were caught on the east side as on the north. Following this, the movement from the north again became the greatest.

For the entire season, the greatest number of weevils were removed from the north part of this trap. In another field a majority were found on the south section of the screen, in two on the east, in two others on the west, while in two fields the numbers were too small to warrant drawing definite conclusions.

### SUMMARY

The cotton-boll weevil has a pronounced habit of dispersal by flight during the summer months, either from one part of a field to another or between fields. Flying began on July 17 in 1925, but not until August 26 in 1924.<sup>6</sup> The weevils commence to fly earlier in

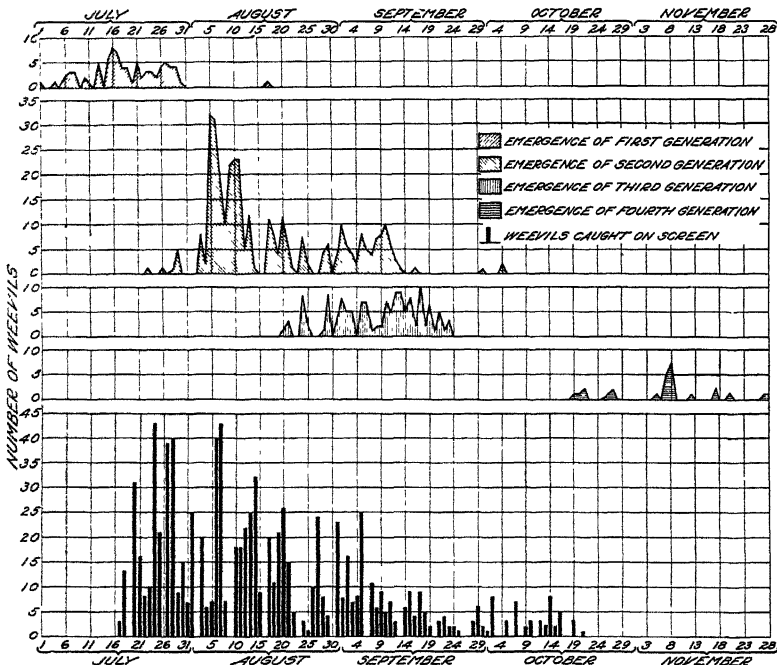


FIG. 9.—Emergence of different generations of boll weevils in 1925 and number of weevils caught on all screen traps

heavily infested fields than in those only slightly infested, the tendency being to leave the former and fly to the latter. Most of these insects fly during the morning, unless it happens to be too cool or rainy, when dispersal takes place in the afternoon, provided the temperature rises, or it clears off. More weevils are in flight at the beginning of the migratory movement than later. Males seem to be more active than females, more being caught on the screens. While the weevils may fly when the maximum temperature is between 60° and 80° F., the most favorable conditions are between 80° and 100° F. There are days when the maximum temperature is favorable, however, and no weevils are in flight. This factor is of secondary impor-

<sup>6</sup> In certain heavily infested fields, weevils were flying before this date.

tance and acts only after the impelling instinct to fly has been awakened.

Such factors as degree of minimum relative humidity, number of squares on the plants, number of weevils in the field, direction of moderate winds, or emergence of a definite generation of weevils, have little influence on the extended flight activities of this species. There is, however, a distinct relationship between degree of infestation in a field and weevil flights. When the percentage of infestation reaches a certain point, which has not yet been determined, these insects become restless and fly. At first their flights are short ones, from row to row, or from one part of a field to another. Later, however, the more heavily infested fields are deserted for those which have been only slightly infested because of poisoning or a low early infestation by overwintered weevils.

#### LITERATURE CITED

- (1) HINDS, W. E.  
1916. BOLL WEEVIL IN ALABAMA. Ala. Agr. Expt. Sta. Bul. 188, p. [23]-64, illus.
- (2) ———  
[1925]. CONTROL OF THE MEXICAN COTTON BOLL WEEVIL. La. Agr. Expt. Sta. Ann. Rpt. 1924: 18-23.
- (3) ——— and YOTHERS, W. W.  
1909. HIBERNATION OF THE MEXICAN COTTON BOLL WEEVIL. U. S. Dept. Agr., Bur. Ent. Bul. 77, 100 p., illus.
- (4) HUNTER, W. D.  
1909. THE BOLL WEEVIL PROBLEM, WITH SPECIAL REFERENCE TO MEANS OF REDUCING DAMAGE. U. S. Dept. Agr. Farmers' Bul. 344, 46 p., illus.
- (5) ——— and HINDS, W. E.  
1905. THE MEXICAN COTTON BOLL WEEVIL. U. S. Dept. Agr., Bur. Ent. Bul. 51, 181 p., illus.
- (6) ——— and PIERCE, W. D.  
1912. THE MEXICAN COTTON-BOLL WEEVIL: A SUMMARY OF THE INVESTIGATION OF THIS INSECT UP TO DECEMBER 31, 1911. U. S. Dept. Agr., Bur. Ent. Bul. 114, 188 p., illus. (U. S. Congress, 62d, 2d Sess., Senate Doc. 305.)
- (7) ISELY, D.  
1926. EARLY SUMMER DISPERSION OF BOLL WEEVIL WITH SPECIAL REFERENCE TO DUSTING. Ark. Agr. Expt. Sta. Bul. 204, 17 p., illus.
- (8) NEWELL, W.  
1906. THE BOLL WEEVIL. INFORMATION CONCERNING ITS LIFE HISTORY AND HABITS. La. State Crop Pest Comm. Circ. 9, 29 p., illus.





# STATUS OF THE PARASITES OF THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR (SAY), IN PENNSYLVANIA, MARYLAND, AND VIRGINIA<sup>1</sup>

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## INTRODUCTION

It has long been known that parasites play an important rôle in the natural control of the Hessian fly, *Phytophaga destructor* (Say), but most of the accounts so far published have contained little information concerning the relationships of the parasites to their host and to each other. Such knowledge, however, is important, not only in estimating the value of the parasites as a whole in the control of the Hessian fly, but also in determining the relative importance of the individual parasites.

To obtain information of this nature an intensive study of the parasites of the Hessian fly has been carried on for several years in the eastern part of the United States, and it is the purpose of this paper to set forth some of the results of this work.

The data here assembled are from material collected for 10 consecutive years from widely separated localities in the wheat-growing regions of Pennsylvania, Maryland, and Virginia, from Lycoming County, Pa., on the north, to Augusta County, Va., on the south.

## METHODS OF INVESTIGATION

In obtaining the records of the parasites attacking the Hessian fly of the spring generation, certain localities in which normal farm practice is followed were selected from the important wheat-growing counties within the area under observation. One or more collections of infested wheat plants were made from each of these points. These samples were examined in the laboratory, the Hessian fly puparia removed and classified, and the apparently sound puparia reared to obtain the parasites or flies that they might contain. In those cases where more than one collection was made in a locality, the data were consolidated and treated as a single collection. The number of puparia thus assembled from each county averaged 310.

In order that all the species of parasites might have a fair showing, collections made earlier than June 15 were not included in the table of relative abundance, and most of the collections were taken after the first of August. It was found necessary thus to take into consideration the time of collection because of the fact that certain of the parasites, for instance, *Platygaster vernalis* and *P. herrickii*, which oviposit in the egg of the host, obviously enter the host considerably earlier than the other parasites. If collections had been made before

<sup>1</sup> Received for publication Oct. 22, 1927; issued March, 1928.

<sup>2</sup> The authors wish to express their obligations to the late W. R. McConnell and P. R. Myers, by whom these investigations were begun, and who contributed substantially to the accumulation of the data used herein. Credit is also due to the various temporary assistants and collaborators who have given their services to this project from time to time. Appreciation is extended to A. B. Gahan for the original determination of most of the species involved and to C. M. Packard, W. H. Larrimer, W. R. Walton, and J. R. Horton, all of the Bureau of Entomology, for helpful criticisms.

the later ovipositing parasites had entered the host there would have been undue discrimination in favor of those which oviposit early.

These parasites all belong to the order Hymenoptera. Of those mentioned in this paper 3 are serphoids of the genus *Platygaster* and 15 are chalcidoids.

The parasites were reared most successfully in shell vials inserted in plaster-of-Paris blocks. This holder was devised by W. R. McConnell early in the progress of the investigations. It consists of a block of plaster 18 inches long by 8 inches high by  $1\frac{1}{2}$  inches thick, with a substantial bulge at the back. Holes about 15 mm. deep and of sufficient diameter to receive 9 mm. by 33 mm. shell vials are spaced an inch apart each way, 119 in all, over the flat surface of the block. The block stands on one long side, and a shallow trough is excavated along the top. Four vertical holes evenly spaced along the bottom of the trough are drilled slightly more than half way to the bottom of the slab. This allows water poured in the trough to penetrate the plaster quickly and uniformly. The puparia are placed in the vials, 10 to each vial, the mouth of the vial is plugged snugly with a piece of cotton, and this end is fitted into one of the holes in the front of the block.

#### PARASITISM OF THE HESSIAN FLY OF THE SPRING GENERATION

From Table 1 it may be seen that in the territory under observation the average annual parasitism of the Hessian fly of the spring generation was 62 per cent.

It was interesting to observe that the average mortality of the Hessian fly amounted to 96 per cent, which is considerably in excess of the mortality that was positively determined as caused by parasites. There is evidence, however, to indicate that some of this additional mortality was the result of parasitism, the traces of which had become obliterated. This was shown to be the case by dissections of Hessian fly puparia taken from selected areas in fields near the beginning and again near the end of the season. Three such sets of observations were made in different localities and in different years and the results in each case showed a greater total parasitism in the first collection than in the last. At the same time, in the last collection an increase was found in the number of puparia dead from undetermined causes. As the number of individual flies remained the same throughout the season, the total amount of parasitism could not actually have decreased, and the difference must be looked for in unrecognizable material. It might be added that in a great many cases, in the course of examining Hessian fly puparia, traces of dead chalcidoids or *Platygaster* larvae were found in an almost unrecognizable state, which also indicates that some of the unrecognizable material within dead puparia was of such origin.

Table 1 also shows the relative abundance of the 18 species of parasites found to prey on the Hessian fly in this section. Three of these parasites belonging to the genus *Platygaster* of the superfamily Serphoidea are *herrickii*, *hiemalis*, and *vernalis*. The remaining 15 species belong to the superfamily Chalcidoidea. *P. vernalis* was more efficient than any one of the other parasites. The other two *Platygaster*s were of insignificant importance, because *hiemalis* normally attacks only the fall generation instead of the spring generation of the fly, and *herrickii* is prolific only in a more southern climate.

TABLE 1.—Relative value of the parasites of the Hessian fly of the spring generation in the wheat regions of Pennsylvania, Maryland, and Virginia, and their relation to the total mortality of the host, 1915 to 1924

Year	Number of collections	Total number of puparia	Average number of puparia per collection	Parasitism of the Hessian fly by—																								
				Chalcidoids												Platygasteroids												
				<i>Eupelmus allyni</i> (French)	<i>Meteorus destructor</i> (Say)	<i>Pleurotropis epio-</i> <i>nus</i> (Walker)	<i>Meteorus febrile-</i> <i>sus</i> Girault	<i>Tetrastichus carina-</i> <i>tus</i> Forbes	<i>Eupleromatus lin-</i> <i>dropterus</i> (Linde-	<i>Chelonus ele-</i> <i>gans</i> (Dalman)	<i>Centropoda specio-</i> <i>sissima</i> (Girault)	<i>Nemicrogaster ful-</i> <i>vipes</i> (Forbes)	<i>Eupelmus salta-</i> <i>tus</i> (Lindemann)	<i>Polycelis modestus</i> <i>Gahan</i>	<i>Ditropilus aureo-</i> <i>viridis</i> Crawford	<i>Callitula bicolor</i>	<i>Eurytoma phebous</i> <i>Girault</i>	<i>Eurytoma n. sp.</i>	<i>Undetermined chal-</i> <i>cidoids</i>	<i>Total chalcidoids</i>	<i>Platygaster herickii</i> <i>Packard</i>	<i>Platygaster thermalis</i> <i>Forbes</i>	<i>Platygaster vernalis</i> <i>(Myers)</i>	Total parasitism		Hessian flies dead from unrecog-	Total mortality of Hessian flies	
				Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1915	5	1,892	376	1.51	2.79	1.45	0.37	0.25	0.06	0.25	0.02	0.10	0.09	0.11	0.08	0.03	0.01	0.06	16.39	22.99	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1916	7	4,947	707	6.77	6.18	3.59	1.42	1.72	0.01	0.12	0.81	0.10	0.09	0.11	0.08	0.03	0.01	0.06	16.39	22.99	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1917	12	1,658	138	0.63	3.02	3.59	1.32	1.40	0.01	0.12	0.81	0.10	0.09	0.11	0.08	0.03	0.01	0.06	16.39	22.99	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1918	5	1,825	365	5.39	2.97	1.81	1.05	0.02	0.04	0.06	0.02	0.05	0.05	0.05	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1919	16	5,225	327	3.19	5.72	1.35	1.03	0.43	0.04	0.06	0.02	0.05	0.05	0.05	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1920	23	6,278	273	2.93	3.24	1.20	0.82	0.19	0.02	0.06	0.09	0.02	0.05	0.05	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1921	15	5,909	394	3.34	3.33	1.28	0.25	0.70	0.02	0.06	0.09	0.02	0.05	0.05	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1922	15	4,455	287	4.64	6.74	1.27	0.81	0.04	0.02	0.06	0.08	0.07	0.07	0.07	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1923	10	2,852	295	2.82	3.39	0.72	0.09	0.04	0.04	0.04	0.08	0.07	0.07	0.07	0.04	0.04	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
1924	15	3,139	209	4.03	2.64	0.84	0.54	2.07	0.06	0.04	0.11	0.02	0.14	0.02	0.02	0.02	0.01	0.01	25.76	40.92	0.51	34.02	57.01	36.27	93.28	93.28	93.28	93.28
Average	12.3	3,317	310	4.02	4.00	1.13	0.63	0.57	0.03	0.05	0.11	0.02	0.04	0.01	0.01	0.01	0.01	0.01	25.67	36.72	0.68	24.81	61.06	33.91	95.57	95.57	95.57	95.57

Of the chalcidoids, five species were found attacking the Hessian fly every year. These were, in order of importance, *Eupelmus allynii*, *Merisus destructor*, *Pleurotropis epigonus*, *M. febriculosus*, and *Tetrastichus carinatus*. The remaining 10 species were comparatively scarce, and in some years a few of them were not recovered at all.

The percentage of the individual chalcidoids is relative rather than absolute because some parasites had emerged at the time of collection. Their occurrence as such, however, could be determined by the emergence holes and pupal remains. These have been classified in the table under the heading "Undetermined chalcidoids," and when they are added to the chalcidoids which were reared and determined there is shown an annual average total parasitism of chalcidoids of 37 per cent.

In order to check the rearing records by actual field conditions, dissections were made of Hessian fly puparia as they were found in the field. This eliminated any modifying effect which might result from unnatural confinement in rearing cages. The combined results of two such dissection records of 99 Hessian fly puparia taken from a field in Carlisle, Pa., in 1918 showed a total parasitism of 70 per cent and a total mortality of 95 per cent. Six such observations, based on 950 puparia collected from a field in Mount Holly Springs, Pa., in 1919, showed a total parasitism of 71 per cent and a total mortality of 92 per cent. Four such observations, based on 586 puparia collected from a field in Mount Holly Springs in 1920, showed a total parasitism of 52 per cent and a total mortality of 99 per cent. Five similar observations, based on 510 puparia collected at New Windsor, Md., in 1921, showed a total parasitism of 53 per cent and a total mortality of 97 per cent. An average of these figures shows a total parasitism of 62 per cent and a total mortality of 96 per cent, which is very close to the average figures obtained in the rearing records shown in Table 1.

#### PARASITISM OF THE HESSIAN FLY OF THE FALL GENERATION

The Hessian fly of the fall generation was found to be attacked almost solely by the serphoid parasite *Platygaster hiemalis*. During the nine years from 1914 to 1922, inclusive, in the wheat-growing regions covered by these investigations, the annual mortality of the Hessian fly of the fall generation caused by this parasite ranged from 16 to 40 per cent with a yearly average of 28 per cent.<sup>3</sup>

Records from material gathered in 10 counties in Pennsylvania, in 1923, showed an average of 55 per cent of parasitism by *hiemalis*. Data obtained during this year from other localities were unreliable owing to the scarcity of the Hessian fly.

#### HYPERPARASITISM

Hyperparasitism is prevalent among the parasites of the Hessian fly, but there is no evidence that any discrimination is made between parasite and fly in the selection of a host.

*Platygaster vernalis* is especially subject to hyperparasitism owing to its occurrence in the host very early in the season. The following parasites have been reared from cocoons of *Platygaster vernalis*: *Eupelmus allynii*, *Merisus destructor*, *M. febriculosus*, *Tetrastichus*

<sup>3</sup> HILL, C. C. *PLATYGASTER HIEMALIS* FORBES, A PARASITE OF THE HESSIAN FLY. Jour. Agr. Research 32: 261-275, illus. 1926.

*carinatus*, *Cheiloneurus elegans*, *Callitula bicolor*, *Nemicromelus fulvipes*, *Eupteromalus micropterus*, *Eupelminus saltator*, *Centrodora speciosissimus*, and *Polyscelis modestus*.

There is evidence which indicates that the chalcidoids prey on one another, as dissection records have shown the presence of more than one chalcidoid larva in a host. Owing to the unrecognizable condition of the parasite killed, it was usually impossible to determine the species and therefore only a few records have been made. *Merisus destructor* was found to have been parasitized by one of its own species and by *Eupelmus allynii*. *Tetrastichus carinatus* was found parasitized by *E. allynii*, *E. allynii* by *T. carinatus*, and an internal parasite considered to be *Pleurotropis epigonus* by *E. allynii*.

The *Platygaster* parasites, *vernalis*, *hiemalis*, and *herrickii*, always oviposit directly in the egg of the fly, and are, therefore, usually primary in their parasitic habits.

It may be assumed that any one of the other parasites is capable of being hyperparasitic. For this reason such parasites as are of little importance as controlling factors because of their scarcity might be considered as detrimental rather than beneficial to the attainment of biological control of the Hessian fly.

#### SUMMARY AND CONCLUSION

The average annual parasitization of the spring generation of the Hessian fly, based on 10 consecutive years of observation, in the region in Pennsylvania, Maryland, and Virginia, from Lycoming County, Pa., to Augusta County, Va., was found to be 62 per cent.

The average total mortality amounted to 96 per cent. The cause of the mortality additional to the 62 per cent mentioned above was unrecognizable at the time of examination. Part of this additional mortality, however, was undoubtedly the result of parasitism.

Eighteen species of parasites were found to attack the Hessian fly in these regions.

The most abundant parasite of the spring generation was *Platygaster vernalis*. Next to this one in order of abundance came *Eupelmus allynii*, *Merisus destructor*, *Pleurotropis epigonus*, *M. jebliculosus*, and *Tetrastichus carinatus*. The remaining parasites attacking the Hessian fly of the spring generation were comparatively scarce.

The fall generation in this region was found to be normally parasitized only by *Platygaster hiemalis*. The average annual mortality of the Hessian fly from this source during the years from 1914 to 1922 amounted to 28 per cent. In 1923 records showed an average of 55 per cent for the State of Pennsylvania.

In this section of the country the two species *Platygaster hiemalis* and *P. vernalis* were the most efficient parasites. *P. hiemalis* should be ranked first in importance owing to its abundance and to the fact that if it were absent there would be no other parasite to attack the fly of the fall generation.

The effectiveness of the remaining parasites, with the exception of *Platygaster herrickii*, is somewhat offset by the likelihood that they may prey on one another and on the *Platygasters*.

Complete control of the Hessian fly by parasites can not be expected, but it is evident that in the regions under observation they are an important factor in checking its increase.



# TAXONOMIC STATUS OF THE DECIDUOUS-FRUIT PARATETRANYCHUS WITH REFERENCE TO THE CITRUS MITE (*P. CITRI*)<sup>1</sup>

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## INTRODUCTION

While making a detailed biological study of what has been held to be the European red mite (*Paratetranychus pilosus* Can. and Fanz.) at the Yakima (Wash.) field laboratory of the Bureau of Entomology, the junior writer became interested in the question whether or not this species is distinct from the citrus mite (*P. citri* McG.) of Florida and California. Opinions have been expressed by various writers on both sides of the question.

The earliest report of the occurrence in America of a *Paratetranychus* on deciduous fruit trees was made by Ewing (6)<sup>2</sup> in Oregon in 1912.<sup>3</sup> He considered the mite found there on apple, peach, and prune to be "the well-known red spider of citrus trees," known then as *Tetranychus mytilaspidis* Riley. Essig (4), in 1913, reported that the citrus mite occurred in California on apple, prune, and peach. Caesar (3) found the deciduous-fruit mite in Canada in 1912, and Frost (7) found it in Pennsylvania in 1918, both of them giving it the name of *P[aratetranychus] pilosus* Can. and Fanz. Garman (8) reported the occurrence of a mite in Connecticut in 1921, "which seems to be *Paratetranychus pilosus* Can. and Fanz.," and, in 1922, Smith (16) reported the European red mite from Idaho, stating that specimens had been determined as such by Ewing.

Garman's article prompted Essig (5) to send specimens of the mites from deciduous and citrus trees in California to Garman, who reported that "no characters could be discovered which would separate the California material from the European red mite." Specimens were also sent to Ewing, who reported that the species occurring on deciduous and citrus fruit trees in California were the same. McGregor (11) showed conclusively in 1916 that the citrus mite was not *Tetranychus mytilaspidis* Riley and that it had never been described. He therefore described it under the name *Tetranychus citri*, and has later placed the species in the genus *Paratetranychus* (12, p. 672). Essig (5), however, concluded that the mite found on both deciduous and citrus trees in California was *Paratetranychus pilosus* and that therefore *P. citri* was a synonym. Subsequent writers have mostly followed Essig's conclusions, in spite of the fact that in 1919 McGregor (12) had described the anatomical differences between *P. pilosus* and *P. citri*.

It seemed to the junior writer that the evidence either for or against the synonymy of these species was not entirely conclusive.

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 180.

<sup>3</sup> Banks (1) described *Tetranychus* (*Paratetranychus*) *bicolor* from oak and chestnut, and *Tetranychus* (*Paratetranychus*) *viridis* from pecan.

The only evidence that existed in favor of the synonymy was a great similarity in appearance and habits, which often occurs with distinct species, and the evidence against synonymy consisted in a difference in food plants and in certain anatomical differences which could be distinguished only with oil-immersion lenses supplemented by keen eyesight, and which were therefore possibly open to question. Correspondence was accordingly begun with the senior writer, with the result that a comparative study of the habits and appearance of the two forms has been made, and the anatomy of each has also been restudied.

#### METHOD OF STUDY

Because the deciduous-fruit mite occurred abundantly at Yakima, Wash., most of the comparative tests were made there by the junior writer. Supplementary tests were also made by the senior writer at the Lindsay, Calif., laboratory of the Bureau of Entomology. The deciduous-fruit mite occurring at Yakima has been determined by Ewing as the European red mite (*Paratetranychus pilosus* Can. and Fanz.), although, as shown later, this determination may be questioned. Through the kindness of R. E. Campbell, of the Bureau of Entomology, United States Department of Agriculture, several shipments of the citrus mite were sent to Yakima on lemon leaves and fruit from Alhambra, Calif. The Yakima experiments were conducted on potted seedling grapefruit trees raised from the seed of Florida grapefruit, on green lemons sent from Alhambra, and on apple, pear, and peach trees planted in the laboratory yard. It is thought that the grapefruit seedlings formed an entirely suitable food plant for the citrus form, because some of the mites were able to maintain themselves on this food from September, 1924, to June, 1925, the seedlings being kept indoors during the winter. In order to keep definite records of individuals, a rearing cell (Fig. 1, B) was used, similar to that perfected by McGregor (13, p. 22) in biological work with *Tetranychus bimaculatus* Harvey. This cell had been used very successfully at the Yakima laboratory in the biological studies of the deciduous-fruit mite. The experiments consisted of (1) mating experiments, both with citrus males  $\times$  deciduous-fruit females, and with deciduous-fruit males  $\times$  citrus females, and (2) feeding experiments in which citrus individuals were reared on deciduous trees and deciduous-fruit individuals on citrus seedling trees or on lemons. A number of minor observations were also made bearing on the problem. Feeding tests only were made at Lindsay, Calif.

#### MATING EXPERIMENTS

The experiments in mating were undertaken because it seemed evident that if these two forms were of the same species they would mate readily and would produce offspring of both sexes, whereas if the two forms were distinct in species such would not be the case. It had been previously established that unfertilized females of these mites deposit eggs normally, and the eggs hatch, but that the resulting mites are always males (parthenogenesis). This was abundantly demonstrated in the course of the biological work with the deciduous-fruit mite at Yakima, and in earlier work by the senior author in South Carolina (13). If the two forms were distinct, the females of either form should produce only males when crossed with males of the other form.



No difficulty was experienced in getting males of either form to mate with females of the other form. Copulation apparently occurred as readily as when the two sexes were of the same form, and lasted about the same length of time. The females used were previously isolated in the deutonymphal stage, so that there would be no possibility of their being fertilized by males of their own form. Males of the other form were placed with them as soon as they cast their nymphal skin. Copulation usually occurred within a few minutes, and care was taken to observe this act and make a record of it. All cases where copulation was not actually observed were thrown out. The females were allowed to remain in the same cell, being always on their native food plant, until they had deposited eggs and had either died or escaped. With a few exceptions each

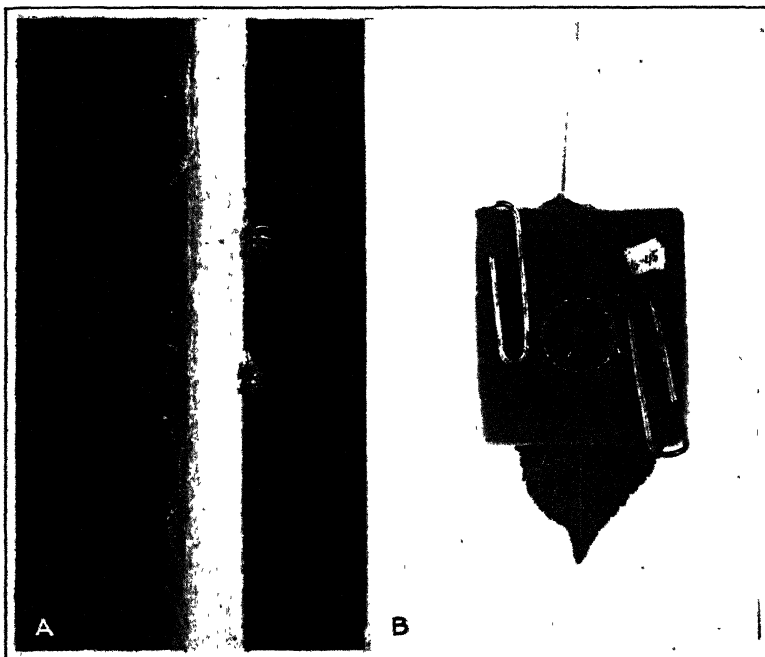


FIG. 1.—Northwestern red mite: A, Adult females on petiole of apple leaf; B, rearing cell used in breeding tests, attached to apple leaf

cell contained only one female, and individual records were made of the life of the female, the number of eggs deposited, and the number and sex of the resulting progeny.

#### CITRUS MALES MATED WITH DECIDUOUS-FRUIT FEMALES

In crossing citrus males with deciduous-fruit females, females were used that had been reared on apple, peach, or pear. This work was started at Yakima in the fall of 1924, but too late to obtain definite results, as cold weather caused the foliage to be shed and put a stop to the development of the eggs and the young. In May and June, 1925, 14 deciduous-fruit females were mated with citrus males; the results of this experiment are given in Table 1.

TABLE 1.—Results of mating male citrus mites with female deciduous-fruit mites at Yakima, Wash., in 1925

No.	Date of mating	Food plant	Length of life of female	Number of eggs deposited	Number of eggs hatched	Number of progeny		
						Male	Female	Not maturing
			<i>Days</i>					
1	Apr. 30.....	Apple.....	27	65	17	12	0	5
2	May 1.....	do.....	12	25	25	13	0	12
3	May 2.....	do.....	15	28	26	25	0	1
4	May 3.....	do.....	20	58	57	45	0	12
5	May 2.....	Peach.....	17	37	34	22	0	12
6	do.....	do.....	40	90	81	45	0	36
7	May 4.....	do.....	13	27	27	27	0	0
8	May 5.....	do.....	24	56	44	36	0	8
9	June 29.....	do.....	9	16	10	10	0	0
10	Apr. 30.....	Pear.....	13	25	25	16	0	9
11	do.....	do.....	25	56	42	9	0	33
12	do.....	do.....	13	9	9	0	0	9
13	do.....	do.....	11	15	15	7	0	8
14	May 1.....	do.....	18	28	19	13	0	6
Total.....				535	431	280	0	151

Average number of eggs per female, 38; percentage of eggs hatching, 81; percentage of eggs producing adults, 52; percentage of young developing into males, 65; percentage of young developing into females, 0; percentage of young not maturing, 35.

Of 535 eggs resulting from these crossings, 431, or 81 per cent, hatched, and 280, or 52 per cent, produced males. No females were produced; and although only a little more than half of the eggs produced males, it does not follow that, if there were any potential females, they were all among the eggs that failed to hatch or among the young that failed to mature. Proof of this statement will be given later. It can only be concluded that these deciduous-fruit females were not fertilized by the citrus males with which they were crossed, and that the young were parthenogenetic. This conclusion is supported by a comparison of these figures with results obtained from deciduous-fruit females mated with deciduous-fruit males at approximately the same time of year. Fourteen citrus  $\times$  deciduous-fruit matings produced 535 eggs, of which 431, or 81 per cent, hatched. Fourteen deciduous-fruit  $\times$  deciduous-fruit matings produced 484 eggs, of which 408, or 84 per cent, hatched. The percentage of hatching is thus very similar. A comparison of the progeny of these two crossings, the latter somewhat amplified in number, is given in Table 2.

TABLE 2.—A comparison of the progeny of female deciduous-fruit mites mated with male citrus mites and with male deciduous-fruit mites, at Yakima, Wash., in 1924 and 1925

Item	Male citrus mite $\times$ female deciduous-fruit mite.		Male deciduous-fruit mite $\times$ female deciduous-fruit mite	
	Number	Percentage of hatching eggs	Number	Percentage of hatching eggs
Eggs hatching.....	431		* 468	
Young failing to mature.....	151	35	100	21
Young developing into males.....	280	65	154	33
Young developing into females.....	0	0	214	46

\* This number includes 60 eggs in addition to the 408 mentioned in the text

Sixty-five per cent of the hatching eggs that resulted from the citrus  $\times$  deciduous-fruit cross produced males, whereas only 33 per cent, or relatively about half as many, of those resulting from the deciduous-fruit  $\times$  deciduous-fruit mating were males. Evidently, in the first case, the females were all replaced by males. Matings of deciduous-fruit males with deciduous-fruit females invariably produce some females, in cases where the number of young maturing is sufficiently large, and usually more females than males.

#### DECIDUOUS-FRUIT MALES MATED WITH CITRUS FEMALES

In the crosses of deciduous-fruit males with citrus females, the latter had been reared on lemons or on grapefruit foliage. Experiments started in the fall of 1924 were carried to completion, since the potted grapefruit seedlings did not shed their foliage, and could be taken indoors. Additional experiments were made in April and May, 1925. Four females were mated in 1924 and six in 1925, as shown in Table 3. The food plant in all these cases was grapefruit foliage.

TABLE 3.—*Results of mating male deciduous-fruit mites with female citrus mites at Yakima, Wash., in 1924 and 1925*

No	Date of mating	Length of life of female	Number of eggs deposited	Number of eggs hatched	Number of progeny		
					Male	Female	Not maturing
	1924	<i>Days</i>					
1	Sept. 12.....	23	12	7	2	0	5
2	Sept. 19.....	16	12	12	12	0	0
3	do.....	17	15	14	12	0	2
4	Oct. 1.....	14	7	5	4	0	1
	1925						
5	Apr. 20.....	18	18	16	14	0	2
6	Apr. 29.....	18	17	17	11	0	6
7	Apr. 30.....	17	13	12	12	0	0
8	May 1.....	16	14	14	4	0	10
9	May 2.....	16	11	11	3	0	8
10	May 3.....	12	8	8	7	0	1
	Total.....		127	116	81	0	35

Average number of eggs per female, 12.7; percentage of eggs hatching, 91; percentage of eggs producing adults, 64; percentage of young developing into males, 70; percentage of young developing into females, 0; percentage of young not maturing, 30.

In this case it is even more conclusive that the young are of parthenogentic origin, since the males constitute 64 per cent of all the eggs deposited, and 70 per cent of the eggs that hatched, and there were no females.

These reciprocal crossings produced 431 hatching eggs from 14 females in one case, and 116 hatching eggs from 10 females in the other case. Only males were produced. This does not happen when females of either the deciduous-fruit or the citrus form are mated with males of their own form. The only conclusion that can be drawn from these experiments is that, although copulation took place, the females were not fertilized, and that therefore the two forms are distinct species.

## MATING EXPERIMENTS WITH DECIDUOUS-FRUIT MITES FROM CONNECTICUT AND WASHINGTON

In the spring of 1926 the junior writer obtained some winter eggs of *Paratetranychus* from Connecticut, through the kindness of Philip Garman, of the Connecticut Agricultural Experiment Station. Mites were reared from these eggs, and matings were made with mites taken from the orchards near Yakima, Wash. Care was observed to use only virgin females. In most cases the males were placed in cells containing only females in the quiescent state following the deutonymphal period. These usually mated as soon as the females emerged. In Table 4 the results of mating nine Connecticut females with Washington males are given. Five of these matings resulted in female progeny, although only 20 per cent of the progeny reared to maturity were females.

TABLE 4.—Results of mating Washington male mites with Connecticut female mites at Yakima, Wash., in 1926

No.	Date of mating	Number of eggs deposited	Number of eggs hatched	Number of progeny		
				Male	Female	Not maturing
1	Apr. 19.....	17	15	1	0	14
2	do.....	8	8	3	2	3
3	Apr. 20.....	7	7	4	0	3
4	do.....	26	23	5	1	17
5	Apr. 22.....	40	12	4	1	7
6	Apr. 24.....	33	18	0	1	17
7	Apr. 25.....	5	4	2	1	1
8	do.....	7	7	3	0	4
9	Apr. 27.....	8	8	2	0	6
Total.....		151	102	24	6	72

Percentage of eggs hatching, 68; percentage of young developing into males, 24; percentage of young developing into females, 6; percentage of young not maturing, 70.

Table 5 presents the results of mating 11 Washington females with a like number of Connecticut males. In seven of these cases female progeny resulted, although only 27 per cent of those maturing were females.

TABLE 5.—Results of mating Connecticut male mites with Washington female mites at Yakima, Wash., in April, 1926

No.	Date of mating	Number of eggs deposited	Number of eggs hatched	Number of progeny		
				Male	Female	Not maturing
1	Apr. 19.....	22	20	4	0	16
2	do.....	18	16	4	0	12
3	Apr. 20.....	15	13	1	0	12
4	do.....	23	22	5	1	16
5	do.....	18	15	3	3	9
6	Apr. 21.....	8	3	0	1	2
7	Apr. 22.....	8	3	0	1	2
8	Apr. 23.....	17	13	5	3	5
9	Apr. 25.....	8	6	1	2	3
10	do.....	15	15	5	0	10
11	Apr. 26.....	13	13	5	1	7
Total.....		173	148	35	13	100

Percentage of eggs hatching, 86; percentage of young developing into males, 24; percentage of young developing into females, 9; percentage of young not maturing, 67.

As a check on these experiments, Connecticut females were also mated with Connecticut males, as shown in Table 6; of four such cases two resulted in female progeny, 13 per cent of the total being females. Seven Connecticut females were isolated and not allowed to mate; Table 7 shows that in none of these cases were any female progeny produced.

TABLE 6.—*Results of mating Connecticut male and female mites, at Yakima, Wash., in April, 1926*

No.	Date of mating	Number of eggs deposited	Number of eggs hatched	Number of progeny		
				Male	Female	Not maturing
1	Apr. 21.....	44	(?)	11	3	(?)
2	Apr. 25.....	61	(?)	9	0	(?)
3	do.....	4	4	3	1	0
4	do.....	4	4	3	0	1
Total.....		113		26	4	

Percentage of mature progeny male, 87; percentage of mature progeny female, 13.

TABLE 7.—*Progeny of unmated Connecticut female mites, isolated at Yakima, Wash., in 1926*

No.	Number of eggs deposited	Number of eggs hatched	Number of progeny		
			Male	Female	Not maturing
1.....	4	4	4	0	0
2.....	9	7	4	0	3
3.....	2	2	1	0	1
4.....	6	5	1	0	4
5.....	18	18	2	0	11
6.....	20	16	1	0	15
7.....	5	4	1	0	3
Total.....	64	51	14	0	37

Percentage of eggs hatching, 80; percentage of young developing into males, 27; percentage of young developing into females, 0; percentage of young not maturing, 73.

Owing to other work it was not possible to follow the development of the progeny of these matings as closely as in the work with the citrus and deciduous-fruit forms. In most cases the experiments had to be discontinued before all of the young had matured, and, since males usually mature more rapidly than females, it is not surprising that a much higher percentage of males, as compared with the percentage of females, resulted than in the other tests.

Since 12 of the 20 reciprocal crossings produced females, it may be argued that the Connecticut and Washington forms are specifically identical. However, as shown later in this paper, preliminary studies of the anatomy of the two forms have failed to corroborate completely the evidence based on the rearing tests. Possibly this should be taken as evidence that the two forms are varietal, instead of being identical species. No difference could be detected in the habits or coloration of the two forms, and certainly they are more closely related than the deciduous-fruit and citrus forms.

## FEEDING EXPERIMENTS

A number of feeding experiments were made to determine whether deciduous-fruit mites would thrive on citrus plants and whether the citrus form would thrive on deciduous plants; and, if these questions should be answered affirmatively, whether the characteristic coloration of each form, which will be described later, would be changed by the new food plant. Mites are not necessarily limited in their feeding habits. The two-spotted mite (*Tetranychus bimaculatus*) and the clover mite (*Bryobia pretiosa*) have a rather large list of food plants. The deciduous-fruit mite has been reported by Garman as found on elm in Connecticut, and the junior writer has found it on elm and on the black locust (*Robinia pseudacacia*) at Yakima, Wash., neither tree being related to its favored food plants, which are in the rose family. The citrus mite does not appear to have been authentically recorded from plants other than citrus. In Europe, *Paratetranychus pilosus* has been recorded by Zacher<sup>4</sup> from 29 host plants, including 11 species of *Prunus*, 3 species of *Pyrus*, 9 species of *Rosa* (the foregoing all in the family Rosaceae), and on *Ribes sanguineum* (Saxifragaceae), *Robinia pseudacacia* (Leguminosae), *Vitis vinifera* (Vitaceae), several species of *Ulmus* (Urticaceae), and a species of *Alnus* (Betulaceae).

The feeding experiments were conducted both in Washington and in California.

## DECIDUOUS-FRUIT MITES ON CITRUS

In the fall of 1924, at Yakima, Wash., 41 newly hatched and day-old deciduous-fruit mites from peach, pear, and apple were placed on green lemons. This was done at various times from August 16 to September 14. Of these, 22, or 54 per cent, lived less than 1 day; 10, or 24 per cent, lived 1 to 2 days; and 9, or 22 per cent, lived 2 to 12 days. Of the latter, 6 reached the protonymphal stage, and the remaining 3 lived until the deutonymphal stage. None matured.

Thirteen deciduous-fruit females from peach and apple were placed on lemons, 5 of them living less than a day, 2 living 1 to 2 days, and 6 living 2 to 9 days. Only 12 eggs were deposited, of which 8 hatched, but none of the young lived more than 2 days. Several attempts were made in 1923 and 1924 to colonize deciduous-fruit mites on the grapefruit seedlings, but without success. Invariably the mites disappeared within a week or so.

On several occasions active stages and eggs of the deciduous-fruit mite were sent from Yakima to Lindsay, Calif., where the senior writer used them in rearing experiments.

On August 3, 1924, living larvae and eggs of the deciduous-fruit form were received at Lindsay and immediately placed on an isolated branch on the south side of a navel orange tree. The eggs continued to hatch, and for a few days larvae and first-stage nymphs were observed on the citrus foliage. All of these died before reaching the second nymphal stage.

On August 10, 1924, a second consignment of material was received from Yakima, and was promptly isolated on a branch on the north side of a vigorous navel orange tree at Lindsay. This lot behaved exactly like the preceding one. Individuals, none of which appeared

<sup>4</sup> Through correspondence with the senior writer.

to be feeding, were seen the first two days moving about on the orange foliage. After an interval of four days no living mites could be found, even by the most critical search.

On September 23, 1924, a colony consisting of a male, a female, and 23 progeny (ova and larvae) was received from Yakima, and these were carefully inclosed within one of the regulation mite-rearing cells attached to an orange tree. The female died September 24, and on the same day 11 ova had hatched and 3 dead larvae were seen. By the 28th one additional egg had hatched, but not a single living mite was present. On the 29th four more ova had hatched, but all active individuals had died. On October 7 one additional egg had hatched and died. On October 14 no more ova had hatched. The weather had been very favorable throughout the rearing period.

On April 8, 1925, a rearing cell was started on a small orange tree, with individuals of the deciduous-fruit mite just received from Yakima. On April 9, six active larvae were present; on the 10th, no larvae had died and no additional eggs had hatched. On the 13th, one larva, one first-stage nymph, and several dead larvae were present; on the 14th, there were one first-stage and one second-stage nymph; on the 15th, all individuals were dead. The temperature had been favorable during the rearing period.

Another rearing cell had been started on April 8, 1925, with Yakima material received that day. Several eggs had hatched by the 9th. By the 10th many eggs had hatched, and all but one larva had died. By the 14th all individuals had died and the colony was extinct.

At the same time that the two last-mentioned cells were started, an apple twig from Yakima, heavily infested with eggs and larvae of the deciduous-fruit mite, was attached to a branch of an orange tree in an orchard at Lindsay. Care had been taken to ring the base of this branch with tanglefoot, and otherwise to isolate it from other parts of the tree. Since this colony existed for about 35 days its daily progress will not be recorded, but the results will be briefly summarized. On April 13 numerous active larvae were seen on the isolated branch, all being on the upper surface of the leaves. On the 14th a few first-stage nymphs had appeared. On the 15th several second-stage nymphs were seen. Many dead individuals were observed on the 16th. A few adult males and females were seen on the 17th. On the 20th a few eggs had been deposited, mostly on the stems of the current year's growth. On the 27th a colony of one female and five eggs was seen. On the 28th another colony, of 35 eggs, was seen. On May 2 this colony consisted of 37 ova and 2 dead larvae. On the 4th several eggs and a few larvae were seen on the isolated branch. On the 13th only five ova could be found and they appeared nonviable. After long search one first-stage nymph was found. On May 22 a most careful search of the isolated orange branch failed to reveal the presence of a solitary individual of the deciduous-fruit mite. No individuals of the second generation seem to have passed the first nymphal stage.

In the course of the rearing tests here recorded no predatory insects or mites were observed. The weather for the most part was very favorable. The death of the colonies should be attributed, it would seem, to inability of the deciduous-fruit mites to adjust themselves to the orange foliage.

The characteristics of the mites reared at Lindsay on citrus agreed with the description of the mites in the Northwest as recorded herein by the junior writer. Furthermore, many eggs were examined and were found to be typical of the eggs of the deciduous-fruit mite, the apical stalk being devoid of any trace of guy fibrils. Obviously the citrus environment had not the slightest effect on the characteristics of any stage of the deciduous-fruit mite.

On June 17, 1926, at Berkeley, Calif., the writers observed a few young mites and eggs on citrus trees on the grounds of the University of California. These trees were within a few feet of apple and plum trees that were heavily infested with the deciduous-fruit mites. After careful observations the writers concluded that the slight infestation on the citrus trees was due to stragglers from the deciduous trees. It was evident that females from the deciduous trees had wandered or been carried to the citrus trees, and that they had deposited eggs there, which had hatched, but that the resulting young were not able to live to maturity. If the deciduous-fruit and citrus forms were identical it would be extremely improbable that the citrus trees in this case would be almost free of the mites when deciduous trees within 10 or 15 feet were very heavily infested.

Poutiers (14) mentions finding *Paratetranychus pilosus* on lemon at Mentone, France, but this may be questioned, as it was not stated that the mites were authoritatively determined.

#### CITRUS MITES ON DECIDUOUS FOLIAGE

Attempts at colonizing citrus mites on deciduous foliage were more successful. On September 8 to 11, 1924, at Yakima, Wash., 81 newly hatched young from lemons were placed in cells on apple foliage. Twenty-six of these, or 32 per cent, lived less than a day; 14, or 17 per cent, lived 1 to 2 days; and 41, or 51 per cent, lived from 2 to 28 days. Twenty of these, or 25 per cent of the total, matured and produced 3 males and 17 females. These females lived from 1 to 26 days, and deposited 40 eggs, none of which hatched, probably on account of the lateness of the season.

On September 23, 15 newly hatched young from lemons were placed in cells on peach foliage. Six of these, or 40 per cent, lived less than 3 days, and 9 lived from 17 to 34 days. Eight of the latter, or 53 per cent of the total, matured and produced females. As this occurred in October the leaves soon dropped, and the length of life of these females was not recorded.

In 1925 further experiments were made at Yakima with larvae from lemons. The larvae were placed in cells on apple, pear, and peach foliage, and records were kept of the number living less than one day, the number living longer but failing to mature, and the number maturing. These records and those obtained in 1924 are shown in Table 8. Parallel records of citrus mites and deciduous-fruit mites on pear and apple are given for purposes of comparison.

These records indicate that the citrus form will live on deciduous foliage as well as will the deciduous-fruit form, at least for the first generation. The high death rate on the first day is largely due to injuries received in the delicate operation of transferring the mites. Of the 30 citrus mites maturing in this experiment 9 were males and 21 were females.



TABLE 8.—*Longevity of larval citrus and deciduous-fruit mites, fed on deciduous foliage at Yakima, Wash., 1924-25*

Food plant	Form	Number of larvæ	Living less than 1 day		Not maturing		Maturing	
			Number	Per cent	Number	Per cent	Number	Per cent
Apple.....	Citrus.....	18	5	28	3	17	10	55
Do.....	Deciduous fruit.....	37	10	27	4	11	23	62
Pear.....	Citrus.....	18	4	22	0	0	14	78
Do.....	Deciduous fruit.....	49	13	27	7	14	29	59
Peach.....	Citrus.....	13	4	31	3	23	6	46
Do.....	Deciduous fruit <sup>a</sup> .....							
Total.....	Citrus.....	49	13	27	6	12	30	61
	Deciduous fruit.....	86	23	27	11	13	52	60

<sup>a</sup> No record.

The 21 citrus females produced on apple, pear, and peach were mated with citrus males, with the exception of two, reared on peach, which were not mated. Records, presented in Table 9, were then kept of the progeny of these females.

TABLE 9.—*Record of second-generation citrus mites, produced on deciduous foliage at Yakima, Wash., in 1925*

Food plant	Cell No.	Number of females in cell	Number of eggs deposited	Number of eggs hatched	Number of young matured
Apple.....	10	1	0	0	0
Do.....	11	3	20	(a) 1	b 1
Do.....	12	1	(a) 3	3	c 1
Do.....	18	1	5	1	d 0
Do.....	19	1	26	8	b 4
Do.....	13	2	(a) 6	6	0
Pear.....	14	1	1	1	c 1
Do.....	15	3	(a) 10	10	0
Do.....	15	1	(a) 9	9	0
Do.....	17	1	(a) 10	10	0
Do.....	21	1	16	10	0
Peach.....	7	1	20	(a) 0	0
Do.....	8	2	21	(a) 0	b 1
Do.....	9	1	8	0	0
Do.....	16	1	1	0	0
Do.....	20	1	15	5	d 2
Estimated total.....			200	85	10
Recorded total.....			133	53	10

<sup>a</sup> Several.<sup>b</sup> Female.<sup>c</sup> Male.<sup>d</sup> 1 male, 1 female.

The junior writer was away from Yakima for a time while these experiments were in progress, and it was therefore not possible in all cases to record the length of life of the females or the exact number of eggs deposited. The maximum number deposited by one female, however, was 26, and the average probably about 10, whereas the average number of eggs deposited by deciduous-fruit females on deciduous foliage at this time of year, as determined at Yakima in 1923 and 1924, was approximately 42. Quayle (15, p. 489) states that the average number of eggs deposited by the citrus mites on citrus is about 30. In most cases the number of eggs hatching was recorded. The total number was estimated at 85, or less than 50 per cent of those deposited. This estimate may be high, for in the cases where com-

plete records were obtained 72 eggs were deposited and 25 hatched, or only 35 per cent. Eggs deposited by deciduous-fruit females on deciduous foliage hatch much better. In 1923 and 1924 records were obtained in the months of May and June of 4,226 eggs of the deciduous-fruit mite; of this number 3,383, or 80 per cent, hatched. These eggs were deposited in cells similar to those used to confine the citrus females.

Of the estimated 85 young hatching, only 10 matured, 3 of them being males and 7 females. Of the 53 young actually recorded only 8 matured, or 15 per cent. These data show a very high death rate; much higher than was found in the first generation, where 61 per cent matured, in spite of the fact that the latter were all transferred by hand from one food plant to another, whereas the ones here considered were allowed to remain in the cells in which they were hatched. This death rate is also much higher than occurs with deciduous-fruit mites on deciduous foliage, even when reared for several generations. The continued feeding on deciduous foliage apparently weakens the citrus form.

The seven second-brood citrus females produced on apple and peach foliage were mated, two of them with citrus males and the others with deciduous-fruit males, no more citrus males being available at this time. Two of the seven females were accidentally killed before depositing all their eggs, and the other five died after depositing only 60 eggs, or an average of 12. Twenty of these eggs, or 33 per cent, hatched. Only 3 of the 20 young matured, or 15 per cent. These are approximately the same percentages as obtained for the second-brood eggs. Unfortunately, the three adults were all males. They were the progeny of the matings of citrus females with deciduous-fruit males, the citrus  $\times$  citrus matings producing no adults. The experiment could therefore be carried no further.

A careful comparison was made of the color of all the citrus mites reared on deciduous foliage. Invariably the color remained characteristic of the citrus form, even to the third generation. This was true of males and females alike, and is considered the most significant part of the feeding experiments. Aside from this, although the citrus form apparently thrives on deciduous foliage during the first generation, it seems to become somewhat weakened, for in succeeding generations a much smaller number of eggs are deposited, a much smaller number hatch, and fewer larvae mature, than is the case with deciduous-fruit mites on deciduous foliage.

In order to show that continued rearing in cells does not materially reduce the oviposition capacity of the females, Table 10 is presented. This table records all of the cases in 1924 where a complete record was obtained of the number of eggs deposited in rearing cells by direct progeny of first-brood mites through four to six generations. Each case represents the number of eggs deposited by a single female in each generation. The high rate of oviposition in the second generation was very noticeable, and is believed to be due to specially favorable weather conditions. These eggs were laid in May and June, just when the citrus mites were being reared, and yet the latter showed no such rate of oviposition. Hot weather later reduced the oviposition rate of the deciduous-fruit females.

TABLE 10.—Records of oviposition of deciduous-fruit mites on deciduous foliage, reared through succeeding generations, at Yakima, Wash., in 1924

No.	Number of eggs deposited by—					
	First generation	Second generation	Third generation	Fourth generation	Fifth generation	Sixth generation
1.....	30	a 5	59	a 20	42	-----
2.....	34	87	a 36	10	a 17	-----
3.....	84	52	a 22	43	49	50
4.....	84	52	a 22	35	a 21	-----
5.....	30	91	a 35	35	a 24	-----
6.....	46	62	31	33	a 28	-----
7.....	46	62	a 33	32	a 14	-----
8.....	a 14	73	40	a 22	-----	-----
Average, including incomplete colonies.....	46	61	35	29	28	-----

a Female was accidentally killed, or escaped, before completing oviposition.

The percentage of eggs hatching is also apparently reduced to some extent by hot weather and increases again in the fall, as shown in Table 11, but in no case is it as low as that of eggs deposited by citrus females reared on deciduous foliage.

TABLE 11.—Records of hatching of succeeding generations of eggs of the deciduous-fruit mite on deciduous foliage, at Yakima, Wash., in 1924

Generation of females depositing eggs	Month in which eggs were laid	Number of eggs laid	Number of eggs hatched	Percentage hatched
First.....	May.....	1, 120	955	85
Second.....	May-June.....	1, 547	1, 246	81
Third.....	June-July.....	1, 152	745	65
Fourth.....	July-August.....	674	494	73
Fifth.....	August-September.....	662	587	89

As already mentioned, red spiders of several species are known to have various food plants, and it is not surprising that the citrus form should be able to develop for a time on deciduous foliage. Essig (5) mentions sending to Garman, for comparison with the Connecticut form, mites that were taken on almond and citrus trees at Berkeley. Essig does not mention any difference in their appearance, and it is possible that all the mites he collected were of one form, especially if two kinds of host plants were growing near each other. It is also possible that two species were represented, and that the gross characters—i. e., coloration, body outline, and the like—were obliterated in mounting, and the specific differences overlooked in the ultimate examination.

#### COLORATION

The coloration of the deciduous-fruit form is quite distinct from that of the citrus form. The body of the deciduous-fruit female is colored a brownish red. The tubercles and hairs are whitish, contrasting strongly with the body color. (Fig. 1, A.) Citrus females feeding on lemon fruit, where they do not get much chlorophyll, are colored a bright red—almost vermilion. Those feeding on foliage are

darker, owing to the influence of chlorophyll, the color being a velvety, purplish red. The tubercles of the citrus form are invariably of about the same color as the body, and the hairs are light reddish, there being little or no contrast between these and the body.

The deciduous-fruit males are greenish in color, the anterior and posterior ends being fulvous; with age the color changes to orange. The citrus males are dark red (bright red on lemons), the anterior and posterior ends being somewhat lighter. As already mentioned, citrus mites fed on deciduous foliage from the time of hatching retained their characteristic coloration, and the adults that matured, even in the second generations, were indistinguishable from citrus mites reared on citrus foliage. Deciduous-fruit mites fed on citrus foliage also retained the color native to the deciduous-fruit form. The males that resulted from crosses of deciduous-fruit and citrus mites invariably bore the color of males of the form to which their female parent belonged. It is thus evident that the difference in

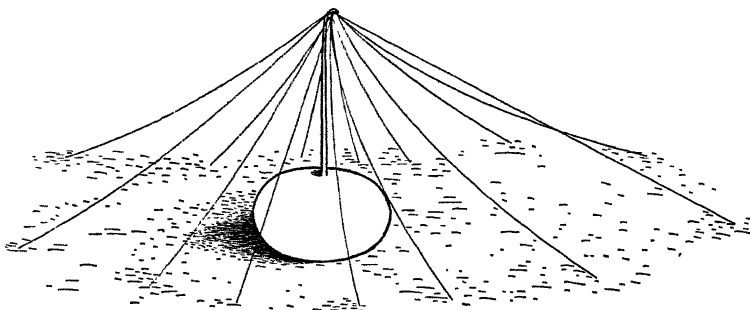


FIG. 2.—Egg of citrus red mite. Method of attachment to leaf is shown. (After Woodworth)

color, as between the two forms, is not the result of feeding on different food plants.<sup>5</sup>

#### OVIPOSITION

The egg of the citrus mite (fig. 2) has been described and illustrated by Woodworth (18), Quayle (15), and McGregor (11), as having webbing or guy fibrils radiating from the egg stalk. The junior writer has never been able to find these guy fibrils on eggs of the deciduous-fruit form, and they are not mentioned in Garman's description of the egg (8). Essig (5) found the webbing on eggs deposited on lemon leaves in California, but considers it "more or less haphazard and due to the number of mites walking over the eggs and the freedom from external influences," although Woodworth and Quayle had previously described the placing of these fibrils by the citrus females.

The junior writer was fortunate in being able to observe several times the act of oviposition by the citrus mite. On September 12, 1924, a citrus female was found ovipositing. As soon as the egg was deposited, the abdomen was gradually elevated until the egg stalk was formed. The female then turned around, applied her

<sup>5</sup> Basing his judgment on a very intimate knowledge of the citrus mite in Florida, and on previous publications on *Paratetranychus pilosus*, W. W. Yothers, of the Bureau of Entomology, at Orlando, Fla., has always maintained that the two species are distinct. After a very extended trip through the Pacific coastal region he wrote to the authors as follows: "While at Yakima, Mr. Newcomer showed me the species *P. pilosus*. It was very evident to an entomologist with a farmer's mind that this was not the same species as *cutri*. After seeing both species, to me it seems inexcusable that anyone should call them the same pest."

mouth parts to the tip of the egg stalk, and attached a thread to it, apparently by rubbing the mouth parts across the end of the stalk. The mouth parts were then moved down to the leaf at one side of the egg, and the thread attached. This performance was repeated ten or a dozen times, each alternate thread usually being attached to the leaf on the side opposite to the point of attachment of the one just before it.

On September 20 another female was observed to deposit an egg, and only five guy fibrils were placed. On November 7 a third observation was made. In this case the female had placed but two fibrils when she was interrupted by the attentions of a male that happened along. The female ran away, but soon returned, and placed eight more fibrils. This action indicates a definite instinct on the part of the citrus female to attach these supports. On November 20 another observation was made, when the female placed six guy fibrils on the egg stalk.

Oviposition by deciduous-fruit females has been observed many times by the junior writer and by M. A. Yothers, of the Yakima laboratory. These females have never been seen to place guy fibrils on their eggs. The process of oviposition is much like that of the citrus form, except that as soon as the egg stalk is made the female runs away and begins feeding, and pays no more attention to the egg. A large number of deciduous-fruit mite eggs have been examined with compound and binocular microscopes in a strong light, and no evidence of these guy fibrils has been found. Occasionally a few strands of webbing would lie upon the egg stalk, but these were evidently made accidentally by other mites that happened to run over the eggs. Although guy fibrils are normally deposited by the citrus females, it is possible that these females do not always place them, and it is also likely that they may become removed when mites or insects run over the eggs; certainly a lack of them is not normal.

The citrus mite apparently does not deposit "winter eggs," as its food plants are in foliage the year around, and the climate in the citrus-growing region is mild enough to enable the mites to develop throughout the year. The deciduous-fruit form, on the other hand, must hibernate because its food plants are without leaves in winter. It does this by depositing "winter eggs" on the twigs, late in the summer. These eggs are slightly larger than the summer eggs, and have a thicker shell. The citrus mites reared on deciduous foliage at Yakima in the fall of 1924 showed no evidence of depositing this special type of egg. The eggs deposited all died on account of cold weather.

#### ANATOMY AND TAXONOMY

Concerning the taxonomic status of the American species of *Paratetranychus*, as based on anatomy, there has been considerable argument in the past. The structures of these mites on which species depend are so ultramicroscopic that actually the question becomes largely a matter of keen eyesight supplemented by a large degree of patience and an ample supply of material. A further handicap in the clarification of the issue is to be found in certain discrepancies and confusion existing in the European literature on the subject.

The original description of *Tetranychus* (*Paratetranychus*) *pilosus* by Canestrini and Fanzago, published 40 years ago, is so brief that it contains little of value to present-day taxonomists.

In 1917 Banks (2) attempted to replace Zacher's generic name *Paratetranychus* with *Oligonychus* of Berlese. In this, Banks seems to have been unjustified, and the writers agree with Zacher (20, p. 91), who presents the facts as follows (translation):

Banks recently designated my genus *Paratetranychus* as a synonym of *Oligonychus* Berlese. I can not agree with this view, since Berlese clearly referred the type species of my genus (*P. pilosus* C. and F.) not to *Oligonychus*, but to *Tetranychus*, and cited specifically *O. brevipodus* Targ.-Tozz. as the one species of *Oligonychus*. From the brief characters of the genus *Oligonychus* very little is to be learned as to whether they agree with the vital characters of *Paratetranychus*, namely, the presence of a claw with empodial appendages shorter than the claw; and with a collar-trachea that is straight and with a bladder-like enlargement at its end. *Oligonychus* appears rather to correspond to *Neotetranychus* Träg. Therefore I hold fast to the designation of *Paratetranychus* for the native species *pilosus* C. and F. and *ununguis* Jac.

As already pointed out, Essig (5) in 1922 attempted to replace *Paratetranychus citri* McG. by *P. pilosus* Can. and Fanz., having been convinced by Garman's and Ewing's diagnoses that both the red mite of the Northwest and the citrus mite of Florida and California were none other than the European red mite. In reaching this conclusion these workers have ignored certain rather evident facts, previously published. In 1915 Trägårdh (17), of Sweden, presented a very detailed account of some of the European tetranychids, accompanied by many rather careful drawings. Among other characters, the structure of the tarsal appendages, the collar trachea, and the penis of *P. pilosus* were presented quite clearly. In addition, Zacher, of Germany, on repeated occasions has published critically on the red spiders of Europe. On most of the anatomical features of *P. pilosus* these two authorities are agreed. This is true of the gross body characters, the palpi, the collar, trachea, and the tarsal structure. They disagree on the outline of the penis.

In connection with the present studies the senior writer has had considerable correspondence with Zacher, who has furnished material of *Paratetranychus pilosus*, and in addition has personally contributed drawings and detailed descriptions. Through the material sent by Zacher from Berlin, Germany, the writers have been able to make a study of the European red mite on apple and on elm. In most respects they have been able to corroborate the published statements of Zacher and of Trägårdh. Unfortunately no males were included in the material from Germany, and it has been impossible to check on the alleged character of the penis of *pilosus*.

At the outset it may be well to state that extended study of the tetranychids has established the fact that, for any given species, most specific characters are somewhat variable. This is true of the palpus, tarsus, penis, collar trachea, and other structures. Of the characters used taxonomically, probably the penis varies least and the tarsus most.

The European authorities seem agreed that the tarsal claw of *Paratetranychus pilosus* possesses only four appendiculate spurs. The writers' studies of European material have revealed that cases occur of tarsi having from four to six spurs, but with the four-spurred claw seemingly typical. In the case of both the citrus mite and the

deciduous-fruit mite of the Northwest the tarsal claw possesses typically six appendiculate spurs.

The collar trachea of *Paratetranychus pilosus* extends ventrad from the middorsal pore as a comparatively short and straight tube, ending abruptly in a semispherical chamber. The collar trachea of both *P. citri* and the northwestern mite is distinctly undulating, somewhat longer, and often terminates in a chamber which is merely a slight enlargement of the tube.

Zacher (19) mentions, and Trägårdh (17, p. 27) and Hirst (10, p. 59) picture, in the female, the terminal "finger" of the palpal "thumb" of *pilosus* as distinctly spatulate—almost as thick as long. In this character the deciduous mite of the Northwest conforms rather closely. The terminal palpal "finger" of *citri* is not strongly spatulate and is decidedly longer than thick.

Concerning the penis structure of *pilosus*, Trägårdh and Hirst agree fairly closely, but the delineation of this organ by Zacher shows certain discrepancies. Trägårdh (17, p. 27) and Hirst (10, p. 51) figure for the penis the following characters: A dorsally situated obtuse basilar lobe; shaft narrowed posteriorly and bent upward to form the tapering hook, which is pointed terminally. A drawing of the penis of *pilosus* by Zacher, made especially for the writers, shows no projection at the usual position of the basilar lobe, but has a dome-like swelling ventrally at a point opposite the usual location of the basilar lobe. (It has been suggested that Zacher's preparations were distorted by treatment with caustic soda.) The *pilosus* type of penis (of Trägårdh and of Hirst) superficially resembles that of *citri* and of the deciduous-fruit form of the Northwest, but both of the American species exhibit penis features which are seemingly distinct. The penis in each case has an acutely pointed basilar lobe, and the distal half of the hook is more acuminate than in *pilosus*. The chief difference between the penis of *citri* and that of the northwestern form lies in the fact that in the former the shaft is shorter than the thickness in the region of the basilar lobe, whereas in the case of the deciduous-fruit form the shaft is longer than the thickness at the basilar lobe.

In the matter of the relative length of the joints of the foreleg, there again seems to be a difference between *pilosus* and the deciduous-fruit form of the Northwest. In the latter the length of the femur equals that of the tarsus; in *pilosus* the length of the tarsus exceeds that of the femur by nearly one-fourth of the latter; in the case of the citrus mite of southern California the reverse seems to be the case, the femur somewhat exceeding the tarsus in length.

It is doubtful if, in most cases, the mandibular plate should be accorded much taxonomic importance. Both emarginate and non-emarginate cases occur in both *pilosus* and *citri*. The latter has a mandibular plate that usually, as a type, has a slight emargination anteriorly; the former is generally without a trace of emargination.

All that should be necessary to dispel any remaining doubt as to the specific distinction between the deciduous-fruit red mite and the citrus mite of America is the very different structure of the eggs of the two species. At different times in the literature, since the first careful description of the citrus mite and its egg, various workers have detailed the unusual structure of the latter. The egg of the

citrus mite is subspherical, being noticeably flattened vertically, and is red in color. From the dorsal "pole" of the egg there arises a stalk which in position is coincident with the continuation of the axis of the egg. The stalk is relatively stout and in length is about twice the vertical diameter of the egg. As a normal feature of oviposition, the female attaches a series of guy fibrils which radiate downward from the tip of the stalk to the supporting leaf surface. (See fig. 2.)

The structure of the egg of the deciduous-fruit red mite of the Northwest is quite different from that of the citrus mite. In outline the egg is spherolenticular, even more flattened than in the case of the egg of the citrus mite, and is red in color. The apical stalk is present, but only slightly longer than the axial diameter of the egg; rarely coincident with the continuation of its axis, being deflected and bent at various angles, and relatively weak. The junior writer has observed many cases of oviposition by the deciduous-fruit mite, but has never seen the formation of guy fibrils, and has no evidence of their existence in this species.

The various writers who have described the egg of the *Paratetranychus* of the eastern part of the United States have failed to mention the presence of the guy fibrils on the axial stalk. Garman (8, 9) published a good figure of the egg, which is devoid of guys. This would suggest an affinity with the northwestern deciduous-fruit mite, but other characters, such as the tarsus and penis, indicate an even closer relationship with the true European form. Certainly the mite of Connecticut is entirely distinct from *Paratetranychus citri* McG.

The egg of *Paratetranychus pilosus* is described by Trägårdh roughly as follows: Roundish;  $29\ \mu$  in diameter, with a short shaft on its under side by means of which it is attached to the supporting plant surface; a groove surrounds the base of the shaft; color yellowish. (Trägårdh does not claim the presence of a dorsal axial stalk.)

#### DESCRIPTION OF PARATETRANYCHUS PILOSUS CAN. AND FANZ.

(Fig. 3)

Length of female 0.315 mm.; color rather uniformly reddish-brown; dorsal bristles stout, pilose, arising from prominent tubercles, the hairs and tubercles of about the same color as the general body color (according to one authority. the bristles arise from whitish tubercles); eye cornea removed from subfrontal bristle a distance about equal to interval between latter and frontal bristle; mandibular plate not emarginate anteriorly; terminal "finger" of palpal "thumb" strongly spatulate, about as wide as long; tarsal joint of foreleg exceeding in length the femur by nearly one-fourth the latter's length; tarsal claw sickle-shaped, nearly as thick at mid-point as at base; at a point not much proximal of the mid-point four appendiculate spurs arise which in length somewhat surpass the claw; collar trachea nearly straight and of even caliber, with spherical dilated terminal chamber; ovum (according to Trägårdh) yellowish, roundish, with a short shaft on its under side by means of which it is attached to plant surface, with a groove around base of shaft; (according to Zacher) red, with a dorsal apical stalk (no mention in the literature of guy fibrils). Penis of male relatively long, with obtuse basilar lobe, shaft nearly half as long as inner lobe, hook bent only  $45^\circ$  from the main axis of penis (according to Zacher, no basilar lobe, but with dome-shaped swelling ventrally). Particularly a pest of fruit trees of the family Rosaceae.



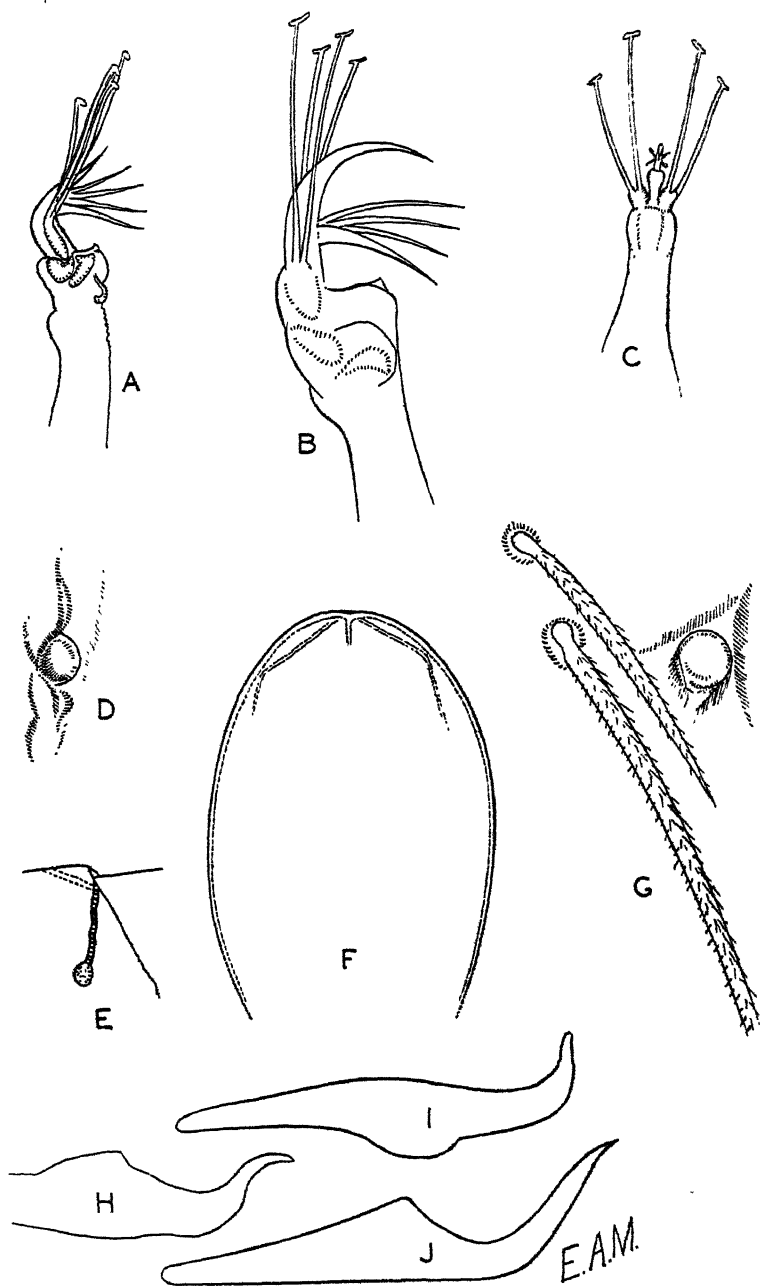


FIG. 3.—*Paratetranychus pilosus*: A, Tarsal appendages viewed in profile (alcoholic material from Berlin, Germany, on apple); B, tarsal appendages viewed in profile (mounted material from Berlin, Germany, on elm); C, tarsal appendages viewed ventrally; D, eye cornea viewed from above; E, collar trachea; F, mandibular plate; G, frontal and subfrontal bristles and eye cornea of right side viewed from above; H, I, J, penis. E and J, after Trägårdh; H, after Zacher

## DESCRIPTION OF PARATETRANYCHUS CITRI McG.

(Figs. 4 and 5)

Length of female 0.305 mm.; color a velvety purplish red, with dorsal tubercles of almost the same color, and with bristles light reddish (tubercles occasionally a little paler than body color); eye cornea about twice as far behind the subfrontal bristle as the latter's distance from the frontal bristle; mandibular plate typically with an inconspicuous emargination anteriorly; terminal "finger" of palpal "thumb" not strongly spatulate, decidedly longer than thick; legs paler than body color; femur somewhat exceeding in length the tarsus; tarsal claw rather straight for two-thirds of its length and then bent sharply downward, noticeably thickest at base, with six appendiculate spurs arising at a point one-third the length of the claw from its base—their tips extending well beyond that of the claw; collar trachea relatively long, undulating, and terminating in a chamber which is often merely a slight enlargement of the tube; ova subspherical, noticeably compressed vertically; at first pale, later turning bright red; the egg bears dorsally an apical stalk which coincides with the continuation of the vertical axis, relatively stout, in length about twice the vertical diameter of egg; normally with a series of guy fibrils radiating from tip of stalk to supporting leaf surface. The color of the male is bright red to dark red, depending somewhat on food plant, the anterior and posterior aspects being somewhat lighter. Penis relatively short, with acute basilar lobe; shaft only about one-third as long as inner lobe, shorter than thickness of penis in region of basilar lobe; hook bent almost 60° from main axis of penis. Almost exclusively a pest of citrus trees.

## DESCRIPTION OF NORTHWESTERN DECIDUOUS MITE

(Fig. 6)

It may be well at this point to record the chief features wherein the deciduous-fruit *Paratetranychus* of the Northwest appears to differ from typical material of *P. pilosus*. These are as follows:

Body color usually garnet-red, dorsal tubercles and bristles whitish; foreleg with femur equaling tarsus; tarsal claw typically possessing six appendiculate spurs; collar trachea often undulating and with terminal chamber elliptical; egg spherolenticular, red, usually with a weak axial stalk slightly longer than vertical diameter of eggs, usually deflected at an angle from the axis of ovum. The color of the male is at first greenish, changing to brick-red or orange; the anterior one-third and posterior tip of body pale pink or fulvous; penis with well developed acute basilar lobe; very sharply acuminate at tip.

Zacher (19) mentions a *Paratetranychus* of a dark cinnamon-brown color with whitish tubercles, occurring in Germany on gooseberry, and, according to Berlese, on pear in Italy. The claw possesses four spurs which are about one and one-half times as long as itself. The penis is broadened spoonlike toward the end, and with a prominent lobe on one side. This he designates as *P. pilosus* var. *alboguttatus*. The coloration of this form considerably resembles the northwestern deciduous-fruit mite, but the other characters, as described by Zacher, are entirely aberrant from the northwestern form.

## CONCLUSIONS

The writers have concluded that the citrus mite (*Paratetranychus citri* McG.) and the European red mite (*P. pilosus* Can. & Fanz.) are entirely distinct species. Practically all the evidence at hand favors this conclusion. It is also concluded that the citrus mite is distinct from the deciduous-fruit forms of *Paratetranychus* found in the United States.

The only evidence indicating that the deciduous-fruit and citrus forms are not more than races of one species is the fact that they are rather similar in general appearance and have similar habits. In

dealing with creatures as small as these one must not be misled by such relatively superficial similarities, but must compare the forms more minutely, both anatomically and biologically. The writers

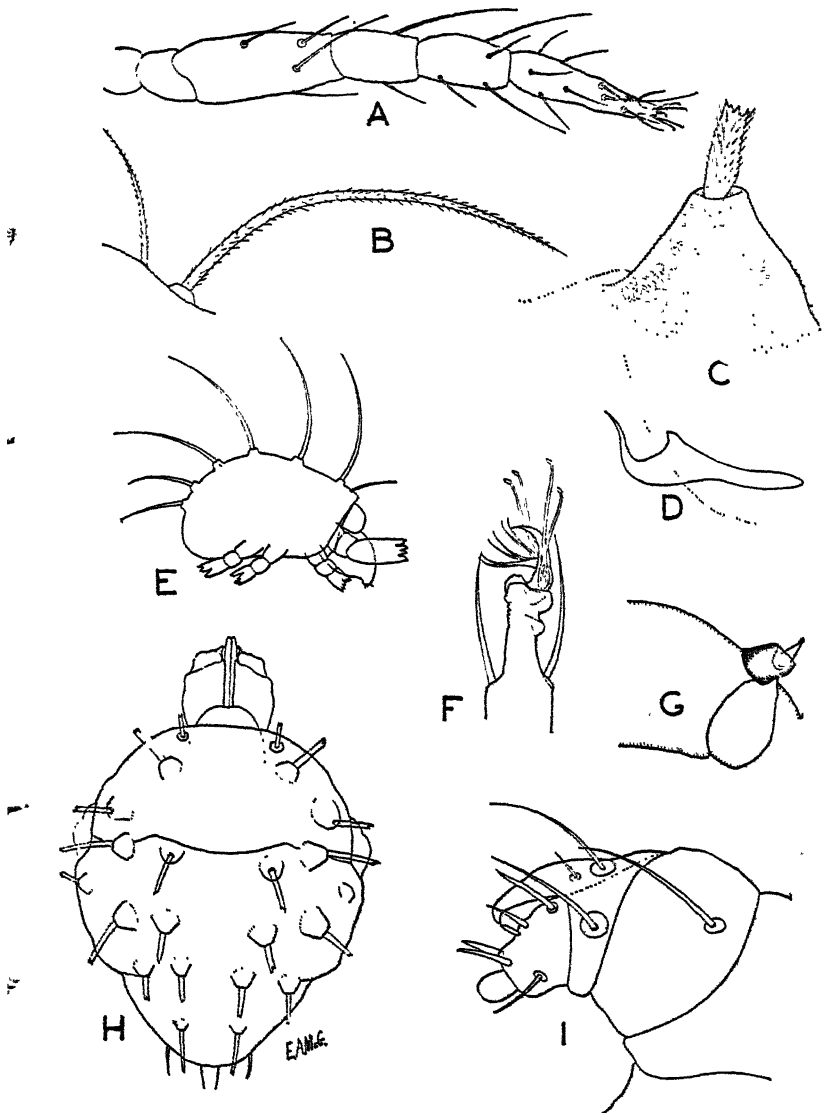


FIG. 4.—*Paratetranychus citri*: A, Right foreleg (dorsal); B, frontal and subfrontal bristles; C, attachment of dorsal bristles to tubercle; D, penis; E, profile view of female (only proximal portion of legs shown); F, tip of tarsus, with appendages; G, section of palpus of male, showing tubercled spur; H, dorsal outline of male, showing distribution of dorsal bristles (the latter amputated); I, left palpus of female (outer view), showing "thumb" claw, and other appendages. Drawn from material from Florida

know from experience that entomologists who have not made a careful study of the biology of mites may very easily arrive at wrong conclusions about them, and it is therefore not at all surprising that such conclusions have been published.

It is believed that the following points give ample evidence that the citrus and deciduous-fruit forms are distinct species.

1. *Anatomical differences.*—Characteristics of *Paratetranychus citri* are: Tarsal "thumb" with terminal "finger" not strongly spatulate, decidedly longer than thick; collar trachea relatively long; femur of foreleg somewhat exceeding the tarsus in length; tarsal claw with proximal two-thirds of length rather straight, noticeably thickest at base; shaft of penis shorter than width in region of basilar lobe; egg subspherical, apical stalk coincident with axis of ovum, relatively stout, about twice the vertical diameter of egg, normally with a series of guy fibrils extending from tip to supporting leaf.

Characteristics of *Paratetranychus pilosus* are: Tarsal "thumb" with terminal "finger" distinctly spatulate, almost as thick as long; collar trachea relatively short; femur of foreleg equaling the tarsus in length; tarsal claw sickle-shaped, about as thick at mid-point as at base; shaft of penis longer than thickness at basilar lobe; egg spherulenticular, apical stalk relatively weak, deflected at various angles from axis of egg, slightly longer than axial diameter of egg, guy fibrils not present.

2. *Failure to interbreed.*—Experiments made by the writers have shown that the progeny of females of either form, when crossed with males of the other form, are invariably all males. Since this occurs when females are not impregnated, it is

evident that these males are produced parthenogenetically, and that no fertilization has taken place.

3. *Failure to thrive on alien food plants.*—The deciduous-fruit mite does not thrive on citrus fruit or foliage, and the writers' attempts to rear it on this food were failures. The citrus form thrives rather well on deciduous foliage for the first genera-

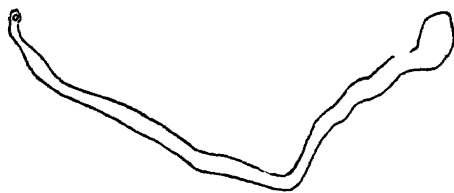


FIG. 5.—Collar trachea of *Paratetranychus citri*. Drawn from material from southern California

tion, but succeeding generations become weakened, depositing only a few eggs, which do not hatch well and the young from which seldom mature. The characteristic coloration of the citrus form is retained, even after two or three generations of feeding on deciduous foliage.

4. *Difference in coloration.*—In color the female of the citrus form is rather uniform purplish red, with concolorous tubercles, and the deciduous-fruit female is a brownish red with discolorous, whitish tubercles. The citrus male is dark red, and the deciduous-fruit male is greenish, turning to orange as it ages. Changing of the food plant fails to alter the respective colors of the two mites. Males resulting from crossing the two forms retain the characteristic color of males of the form to which their female parent belonged.

5. *Different oviposition habits.*—The citrus females deliberately attach their eggs with numerous guy fibrils, radiating from the egg stalk to the leaf. The deciduous-fruit females never do this. The latter deposit "winter" eggs in the fall, whereas the former do not, even under similar conditions.

6. *Different habitat.*—Finally, the habitats of the citrus and deciduous-fruit forms do not overlap. The deciduous-fruit form has not been recorded south of latitude 37° N., either in Europe or America. On the other hand, the citrus form does not seem to occur north of latitude 34° or 35° N. In California, the deciduous-fruit form has not been found south of the San Francisco Bay region, and the citrus form does not occur north of the Tehachapi Mountains. This distribution leaves a gap of about 250 miles. This gap is not caused by lack of host plants, for citrus trees are grown commercially north of Sacramento, often in close proximity to deciduous fruit trees, and various deciduous fruits are grown commercially in or bordering the citrus districts of southern California. The two host plants thus overlap.

On the Atlantic coast, the deciduous-fruit mite has not been recorded south of Charlottesville, Va. (lat. 38° N.), while the citrus mite does not seem to have been recorded north of Florida, there being a gap of about 500 miles between these limits. Deciduous fruit trees are grown in Georgia and in Florida, and to some extent overlap the range of the citrus trees.

In the foregoing discussion of the anatomy and biology of the American species of *Paratetranychus* it is felt that strong evidence has been presented contributing to the solution of their taxonomic status. The writers prefer to abstain from including the several European species of *Paratetranychus* in the treatment set forth here.

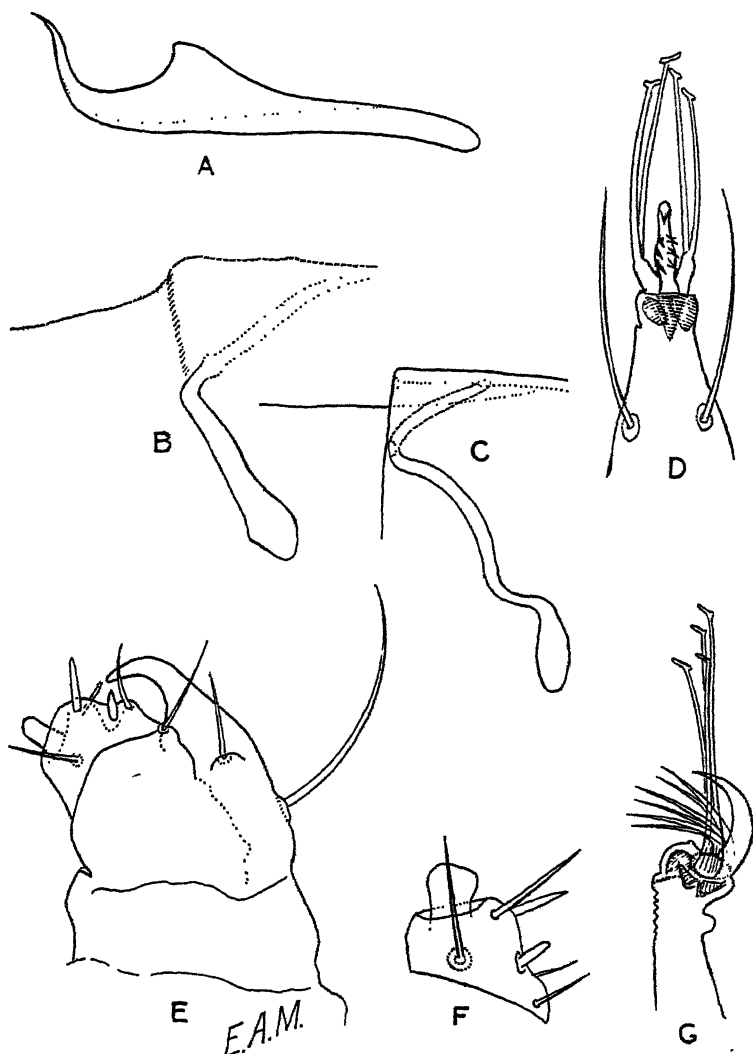


FIG. 6.—*Paratetranychus pilosus* var. *occidentalis*: A, Penis; B, collar trachea; C, collar trachea; D, tarsal appendages, viewed ventrally; E, male palpus and its appendages; F, female palpus and its appendages; G, tarsal appendage in profile. B, Drawn from material from Portland, Oreg.; the other figures drawn from material from Yakima, Wash.

When all the facts are considered, however, it appears that the closest American ally to *P. pilosus* Can. and Fanz. is to be found in the Atlantic coast deciduous-fruit species. The tarsus, penis, collar trachea, and ovum characters of the two latter species seem to agree very closely, and both species appear to feed chiefly on woody species

of the Rosaceae. In comparing the Atlantic species with the red mite of the Northwest, the writers have noted that the evidence is inconsistent; the anatomical characters fail to agree,<sup>6</sup> whereas the rearing experiments suggest that they may be identical.<sup>7</sup> Possibly, for the present, it is best to call the Atlantic species *P. pilosus*, and to designate the deciduous-fruit mite of the Northwest as *P. pilosus* var. *occidentalis*. The complete nonidentity of the citrus and the deciduous-fruit species has been convincingly demonstrated.

## LITERATURE CITED

- (1) BANKS, N.  
1894. SOME NEW AMERICAN ACARINA. Amer. Ent. Soc. Trans. 21: 209-222.
- (2) ———  
1917. NEW MITES, MOSTLY ECONOMIC (ARACH., ACAR.). Ent. News 28: 193-199, illus.
- (3) CAESAR, L.  
1915. AN IMPORTED RED SPIDER ATTACKING FRUIT TREES. Canad. Ent. 47: 57-58, illus.
- (4) ESSIG, E. O.  
1913. INJURIOUS AND BENEFICIAL INSECTS OF CALIFORNIA. THE CITRUS RED SPIDER. Calif. State Comm. Hort. Mo. Bul. 2: 9-10, illus.
- (5) ———  
1922. THE EUROPEAN RED MITE. Calif. Dept. Agr. Mo. Bul. 11: 409-411, illus.
- (6) EWING, H. E.  
1912. THE OCCURRENCE OF THE CITRUS RED SPIDER, TETRANYCHUS MYTILASPIDIS RILEY, ON STONE AND POMACEOUS FRUIT TREES IN OREGON. Jour. Econ. Ent. 5: 414-415.
- (7) FROST, S. W.  
1919. THE IMPORTED RED SPIDER (PARATETRANYCHUS PILOSUS CAN. & FANZ.) ATTACKING APPLE FOLIAGE. Jour. Econ. Ent. 12: 407-408.
- (8) GARMAN, P.  
1921. THE EUROPEAN RED MITE (PARATETRANYCHUS PILOSUS CAN. & FANZ.) IN CONNECTICUT. Jour. Econ. Ent. 14: 355-358, illus.
- (9) ———  
1923. THE EUROPEAN RED MITE. Conn. State Agr. Expt. Sta. Bul. 252, p. [103]-125, illus.
- (10) HIRST, S.  
1920. REVISION OF THE ENGLISH SPECIES OF RED SPIDER (GENERA TETRANYCHUS AND OLIGONYCHUS). Zool. Soc. London, Proc. 1920: 49-60, illus.
- (11) MCGREGOR, E. A.  
1916. THE CITRUS MITE NAMED AND DESCRIBED FOR THE FIRST TIME. Ann. Ent. Soc. Amer. 9: 284-288, illus.
- (12) ———  
1919. THE RED SPIDERS OF AMERICA AND A FEW EUROPEAN SPECIES LIKELY TO BE INTRODUCED. U. S. Natl. Mus. Proc. 56: 641-679, illus.
- (13) ——— and McDONOUGH, F. L.  
1917. THE RED SPIDER ON COTTON. U. S. Dept. Agr. Bul. 416, 72 p., illus.
- (14) POUTIERS, R.  
1921. LE DÉPÉRISSEMENT DES AGRUMES CAUSÉ PAR LES TÉTRANYQUES. Prog. Agr. et Vitic. 76: 117-118.

<sup>6</sup> This statement is based largely on drawings and descriptions by eastern workers.

<sup>7</sup> In response to an inquiry, Burlingame, geneticist at Stanford University, has expressed the following opinion: "If two supposed species can be crossed with some success, they might still be considered as belonging to different species, provided there were other good reasons of convenience for so considering them." He cites the fact that the American bison and the domestic cattle have been successfully crossed. The young from this cross were then bred back to the cow, and the progeny of this latter cross will breed among themselves. The cow and the bison are even placed in different genera by the systematists, and, although they were made to breed together, no one would attempt to place them in the same species. Burlingame adds: "If two supposed species will not cross successfully there is good reason for saying that they are different species."

- (15) QUAYLE, H. J.  
1912. RED SPIDERS AND MITES OF CITRUS TREES. Calif. Agr. Expt. Sta. Bul. 234, p. [483]-530, illus.
- (16) SMITH, R. H.  
1922. SPIDER MITES AFFECTING ORCHARD AND GARDEN FRUITS. Idaho Agr. Expt. Sta. Circ. 25, 8 p., illus.
- (17) TRÄGÅRDH, I.  
1915. BIDRAG TILL KÄNNEDOMEN OM SPINNKVALSTREN (TETRANYCHUS DUF.). Meddel. Centralanst. Försöksv. Jordbruksområdet [Sweden] 109, 60 p., illus. [English summary, p. [53]-59.]
- (18) WOODWORTH, C. W.  
1902. THE RED SPIDER OF CITRUS TREES. Calif. Agr. Expt. Sta. Bul. 145, 19 p., illus.
- (19) ZACHER, [F.].  
1913. UNTERSUCHUNGEN ÜBER SPINNMLBEN. Mitt. K. Biol. Anst. Land u. Forstw. 14: 37-41, illus.
- (20) ———  
1921. UNTERSUCHUNGEN ÜBER SPINNMLBEN. Mitt. Biol. Reichsanst. Land u. Forstw. 21: 91-100.





# CHEMICAL CHANGES IN DUSTING MIXTURES OF SULPHUR, LEAD ARSENATE, AND LIME DURING STORAGE<sup>1</sup>

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United States Department of Agriculture*

## INTRODUCTION

Since dusting for the control of insects and fungous diseases became a commercial practice it has been usual to combine the insecticide and the fungicide in one mixture, and from the economic standpoint this has been a practical necessity. Such a combination is, of course, impossible unless the materials to be mixed are compatible. That is, they must not react with one another in such a manner as to reduce materially either the insecticidal or the fungicidal value of the ingredients, and, in addition, such combination must not increase the danger of injury to the plant to which it is applied.

Compatibility is a relative term and is usually dependent upon a time factor. A combination which is perfectly compatible under experimental conditions, where the ingredients are combined shortly before being used, may prove incompatible under conditions of commercial manufacture.

Under commercial conditions the materials must be combined considerably in advance of an anticipated demand, as most orders are for immediate delivery. This very often results in a surplus over the needs for the current year, which must either be used up the following year or become a complete loss.

The grower, too, must purchase in advance of his needs and often has a considerable quantity left at the end of the season, which he keeps until the next year.

Under such conditions a combined insecticide and fungicide has from several weeks to a year or more in which to react. Very little information has been made available thus far concerning the effects of such long storage, and for this reason the present investigation was undertaken to determine the nature and extent of such changes and their probable effect upon the insecticidal and fungicidal value of the dust.

## THE DUST OF SULPHUR, LEAD ARSENATE, AND LIME

The particular dust dealt with in this article is the 80-5-15, commonly used for the control of insects and diseases on peaches (10).<sup>2</sup> It has the following composition:

	Per cent
Sulphur flour.....	80
Acid lead arsenate.....	5
Hydrated lime.....	15

<sup>1</sup> Received for publication Nov. 16, 1927; issued March, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 191.

The specifications for these ingredients are as follows:

Sulphur: Superfine sulphur flour, 95 per cent passing through a 200-mesh sieve.

Lead arsenate: Standard lead arsenate containing at least 30 per cent arsenic pentoxide, with metallic arsenic in water-soluble form not exceeding 0.75 per cent.

Lime: Hydrated lime of high calcium content analyzing over 90 per cent calcium hydroxide.

#### PURPOSE OF VARIOUS INGREDIENTS

Sulphur is used for the control of fungous diseases attacking fruit. Lead arsenate is used as a general insecticide for the control of chewing-insect pests. Lime is added for the following purposes (4, 5): (1) To improve the physical properties of the dust by eliminating lumping; (2) to make the dust more fluid, so that it is discharged much more uniformly from the duster; (3) to reduce the cost of the material by displacing sulphur; (4) to reduce the arsenical injury to the plant to which it is applied.

#### PHYSICAL CHANGES OF MIXTURE DURING STORAGE

The correct quantities of the three ingredients are put into a revolving drum fitted with baffle plates, and thoroughly mixed. The mixture is then weighed into paper-lined burlap bags and stored in this form till sold. Within a month, and sometimes within two weeks, from the time it is mixed the color of the dust has changed from a light yellow to a light gray. Upon longer storage this shade may deepen to a dark gray or black. One-year-old dusts obtained from various manufacturers and farmers differed greatly in shade, ranging from light gray to black. As a rule, with few exceptions, a dust which has reached only the light-to-dark-gray stage within a year does not darken further during the succeeding year's storage. Whereas a fresh dust is very fluid and quite finely divided, a 1-year-old dust has lost much of its fluidity and is more or less lumpy, thus being less efficient for dusting purposes.

#### PLAN OF INVESTIGATION

The investigation of these changes was carried out along the following lines: (1) Qualitative tests upon old and new dusts to determine what changes had taken place; (2) quantitative analyses to determine the extent and, as far as possible, the mechanism of such changes; (3) probable effect of such changes upon the insecticidal and fungicidal value of the dust; (4) field spraying tests with various compounds formed during storage to determine what effect these would have upon arsenical injury to plants.

#### QUALITATIVE TESTS

These analyses were made according to the methods of Hinrichs (8), and the results were checked by the methods of Chamot (3). The results obtained are given below.

NEW DUSTS.—Contained no sulphides or sulphites and only small quantities of carbonates.

OLD DUSTS.—The gray to black shade was due to varying quantities of lead sulphide formed. Small quantities of sulphites and large quantities of carbonates were present also.

## QUANTITATIVE TESTS

The analytical methods that were employed are described below.

**TOTAL AND WATER-SOLUBLE ARSENIC.**—The official methods adopted by the Association of Official Agricultural Chemists (1) were used.

**SULPHITES.**—The modification of the official method of the Association of Official Agricultural Chemists (1) described below was used.

Place 15 gm. of the dust in a 500 c. c. distilling flask. Add 5 c. c. of alcohol and 20 c. c. of water. Mix thoroughly to wet the dust. Add an additional 200 c. c. of water. Expel the air from the apparatus with carbon dioxide, add 25 c. c. of 20 per cent glacial phosphoric acid, and distill in a constant current of carbon dioxide until 100 c. c. has passed over. Collect the distillate in 200 c. c. of nearly saturated bromine water, allowing the tip of the condenser to dip below the surface.

Boil the distillate to remove excess bromine; acidify with hydrochloric acid and again bring to boil. Then add an excess of 10 per cent barium chloride and boil for a few minutes. Cover with a watch glass and allow to stand overnight.

Filter through a weighed Gooch crucible; wash with hot water. Ignite to dull red heat and weigh as barium sulphate. Express as calcium sulphite.

**SULPHIDES.**—Place 15 gm. of the dust in a 500 c. c. distilling flask. Add 5 c. c. of alcohol and 20 c. c. of water and mix to wet the dust. Add an additional 200 c. c. of water. Allow tip of condenser to dip below the surface of 100 c. c. of 20 per cent sodium hydroxide in a beaker. Add 30 c. c. of concentrated hydrochloric acid to the dust in the distilling flask by means of a dropping funnel. Heat gently until the dark color is completely removed from the dust. Continue heating gently for 15 minutes. Sweep out apparatus with a current of carbon dioxide.

Oxidize sulphides and sulphites in distillate to sulphates by adding 5 gm. of sodium peroxide and boiling for 10 minutes. Acidify with concentrated hydrochloric acid and boil for a few minutes. Add an excess of 10 per cent barium chloride, boil again for a few minutes, and allow to stand overnight. Filter through a weighed Gooch crucible; ignite at a dull red heat and weigh as barium sulphate. Subtract from this the quantity due to sulphites. Calculate as lead sulphide.

**CARBONATES.**—Distill over, as in the case of sulphides, into 100 c. c. of 20 per cent sodium hydroxide. Sweep out apparatus with carbon-dioxide-free air. Transfer distillate to hot plate. Add 5 gm. of sodium peroxide and boil for 10 minutes to convert the sulphides and sulphites into sulphates. Remove, place in a 500 c. c. Erlenmeyer flask, and carry out the determination according to the official method for the determination of carbon dioxide in Bordeaux mixture (1).

**HYDROGEN-ION CONCENTRATION.**—Shake 1 gm. of dust for one minute with 10 c. c. of previously boiled and cooled distilled water. Allow to stand for 10 minutes. Filter and determine  $P_H$  by colorimetric method.

The results of the tests are given in Tables 1 to 5.

TABLE 1.—*Water-soluble arsenic in sulphur-lead-arsenate-lime dusts of various ages*

Sample No.	Water-soluble arsenic pentoxide as per centage of total arsenic oxide		
	Fresh sample	1 year old	2 years old
	Per cent	Per cent	Per cent
1.....		1.20	1.20
2.....	1.16	4.06	
3.....		2.30	2.33
4.....	.55	1.00	
5.....		2.46	2.46

TABLE 2.—*Sulphite content in sulphur-lead-arsenate-lime dusts of various ages*

Sample No.	Sulphite present (as calcium sulphite) per 15 gm. of dust		
	Fresh sample	1 year old	2 years old
1.....	None.	Gram 0.0562	Gram
2.....		.5300	0.531

TABLE 3.—*Sulphide content in sulphur-lead-arsenate-lime dusts of various ages*

Sample No.	Sulphides (as lead sulphide) per 15 gm. of dust		
	Fresh sample	1 year old	2 years old
1.....	None	Gram 0.0261	Gram
2.....		2043	0.2052

TABLE 4.—*Carbonate content in sulphur-lead-arsenate-lime dusts of various ages*

Sample No.	Carbon dioxide content per 15 gm. of dust		
	Fresh sample	1 year old	2 years old
1.....	Gram 0.11	Gram 1.862	Gram
2.....		.755	0.755

TABLE 5.—*Hydrogen-ion concentration in lead-arsenate-sulphur-lime dusts of various ages*

Material	P <sub>H</sub>	Material	P <sub>H</sub>
Freshly prepared dust.....	9.8	Calcium sulphite.....	7.6
1-year-old dust (light gray).....	7.2	Calcium carbonate.....	6.6
1-year-old dust (black).....	9.6	Calcium arsenate.....	9.8
2-year-old dust (black).....	9.4	Water used in above tests.....	6.3

There is a slight increase in soluble arsenic during the first year's storage, as shown in Table 1. Very little or no increase was evident during the second year.

Sulphites, sulphides, and carbonates also increased somewhat during the first year and only very slightly during the second, as shown in Tables 2, 3, and 4.

The hydrogen-ion concentration increased somewhat during storage, increasing in a gray dust much more rapidly than in a black dust, as shown in Table 5. There was also a slight increase in the case of the black dust during the second year's storage. The reasons for this difference will be discussed later. As alkalinity in a fresh dust is due to the lime present, the decrease in  $P_H$  value during storage would seem to be due to the conversion of hydrated lime into calcium sulphite and calcium carbonate. This view is substantiated by the results of analyses and by the  $P_H$  of these salts in solution.

#### REACTIONS INVOLVED IN DARKENING OF A DUST

In an attempt to explain why some dusts darken more than others and the mechanism of the reactions involved, certain mixtures were made up in 200 c. c. of water and boiled. The mixture and the results follow.

Sulphur (C. P.) plus acid lead arsenate (C. P.).....	No change in color.
Sulphur (dusting) plus acid lead arsenate (commercial).	No change in color.
Calcium oxide (C. P.) added to both of above while boiling.	Black lead sulphide formed.
Sulphur (dusting) plus acid lead arsenate (commercial) plus hydrated lime.	Black lead sulphide formed.
Sulphur (dusting) plus acid lead arsenate (commercial) plus calcium carbonate.	No change in color.

From these results it was evident that chemically pure chemicals went through the same reactions, and darkened in the same manner, as the commercial products, thus proving that the reactions were not due to impurities.

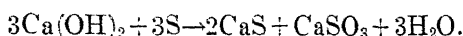
It was also evident that lime played an important part in the formation of lead sulphide and the consequent darkening of the mixture. The addition of calcium carbonate had no effect, a fact noted by Ginsburg (?).

Various dust mixtures were then made up, stored in paper bags in the laboratory for one year, and then examined. The results are given below.

Acid lead arsenate plus sulphur.....	No change in color; no sulphides, sulphites, or carbonates.
Acid lead arsenate plus hydrated lime.	No change in color; large quantity of carbonates.
Sulphur plus hydrated lime.....	Small quantities of sulphides and sulphites; large quantity of carbonates; no change in color.
Acid lead arsenate plus sulphur plus hydrated lime.	Color dark gray; sulphides, sulphites, and carbonates present.
Acid lead arsenate plus sulphur plus calcium carbonate.	No change in color; no sulphides or sulphites.

The same results were therefore obtained in the case of dusts stored for one year as in the case of the boiled mixtures.

The first step, therefore, in the darkening of a dust probably involves the reaction of lime with sulphur to form calcium sulphide and calcium sulphite:

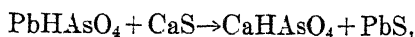


This reaction would result in the formation of two molecules of the sulphide to one molecule of the sulphite. That this is approximately the case was shown by storing a mixture of lime and sulphur in an air-tight container and analyzing after six months. The results are given in Table 6. Complete agreement is impossible as the air can not be completely removed from a fresh dust.

TABLE 6.—*Ratio of sulphide to sulphite produced in stored lead-arsenate-sulphur-lime dusts*

Weight in grams, per 15 gm. of dust		Molecular ratio
Calcium sulphide	Calcium sulphite	
0.040	0.036	1.85 to 1.00

Part of the calcium sulphide formed then reacts with lead arsenate to form black lead sulphide:



thus causing the dust to become gray to black, depending upon the extent of the reaction.

This would result in the formation of calcium arsenate. If this material is formed, there would be expected a very great increase in soluble arsenic upon digestion of the dust with water saturated with carbon dioxide (9). The darker the dust, the more calcium arsenate should be present and the greater the increase in soluble arsenic upon the action of carbonic acid. This was found to be the case, as shown in Table 7. A portion of the calcium arsenate here found is probably due also to direct reaction between the lime and lead arsenate, as suggested by Campbell (2), and possibly some to that between calcium carbonate and lead arsenate, as suggested by Ginsburg (7).

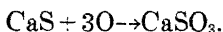
TABLE 7.—*Percentage of soluble arsenic in different colors of sulphur-lead-arsenate-lime dusts digested for six hours at 32° C. in water saturated with carbon dioxide*

Color of dust	Percentage of arsenic pentoxide which is water soluble
Light yellow.....	10.0
Light gray.....	11.9
Black.....	41.5

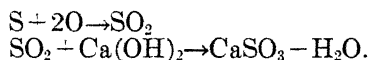
The large quantity of calcium arsenate in a black dust would also explain the difference in  $P_H$  between the light gray and black year-old dusts noted in Table 5, since calcium arsenate has a  $P_H$  approaching that of a fresh dust. Its formation would therefore tend to prevent great changes in the  $P_H$  value.

#### OTHER REACTIONS OCCURRING DURING STORAGE

The calcium sulphide not reacting with lead arsenate is apparently oxidized to sulphites:

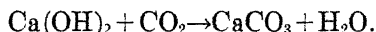


This is indicated by the fact that dusts stored in porous containers show a much larger quantity of sulphites than sulphides, the opposite being the case when stored in air-tight containers. A portion of the sulphites are probably formed directly by the action of sulphur dioxide upon lime:



Further oxidation probably results in the formation of sulphates, but no effort was made to determine this radical, as its formation is of little importance.

One other reaction of considerable importance also occurs, namely, that of the conversion of lime into calcium carbonate:



Moisture and concentration of carbon dioxide are the two main factors influencing the speed of this reaction. Although the concentration of carbon dioxide in the air is fairly constant, the tightness of the container and the porosity of the dust are factors influencing to some extent the concentration of gas available for reaction within the dust. Moisture is apparently the most important factor, the reaction taking place more rapidly under conditions of high humidity.

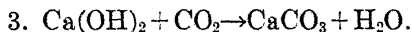
#### FACTORS INFLUENCING THE EXTENT OF DARKENING OF A DUST

The reactions causing the darkening of a dust may be summarized:

1.  $3\text{Ca}(\text{OH})_2 + 3\text{S} \rightarrow 2\text{CaS} + \text{CaSO}_3 + 3\text{H}_2\text{O}.$
2.  $\text{CaS} + \text{PbHAsO}_4 \rightarrow \text{CaHAsO}_4 + \text{PbS}.$

The variables involved in the speed of the foregoing reactions are the concentration of hydrated lime, the temperature, and perhaps to some extent the moisture.

The factors influencing the concentration of hydrated lime at any one time are the quantity originally present and the extent to which the reaction given below has occurred:



It has been previously shown that calcium carbonate will not react with sulphur to form sulphides. Therefore, the extent to which a dust will darken depends upon the relative speed of reactions 1 and 3.

High temperature is the principle factor favoring the former, whereas porosity and high humidity favor the latter.

It would then be expected, and such was found to be the case, that a dust stored under very hot, dry conditions darkens much more quickly and to a much greater extent than one stored under cool, damp conditions.

#### CHANGES IN FUNGICIDAL AND INSECTICIDAL VALUE

The fungicidal efficiency of the dust changes very little, if at all, during storage as the greatest quantity of sulphur undergoing chemical change in any of the dusts tested was less than 0.5 per cent of the total quantity present. This quantity is considered too small to change materially its effectiveness, as Chase (6) records practically as good results in disease control in using 25 per cent less sulphur than is contained in the 80-5-15 formula.

A portion of the lead arsenate is changed during storage into calcium arsenate. As calcium arsenate has proved almost as effective an insecticide as lead arsenate, little or no decrease in insecticidal efficiency should result from storage.

#### FIELD SPRAYING AND DUSTING TESTS

As there was little or no change in insecticidal and fungicidal efficiency during storage, there yet remained to be determined the factor of safety as regards the plant to which the dust may be applied. As it has already been sufficiently proven that injury to fruits and plants is due to the arsenicals used, tests were conducted with calcium arsenate and lead arsenate both alone and in combination with the various buffer salts present in old and new dusts.

These materials were applied for comparative purposes as sprays since this method is much more accurate for small test plots. All materials were applied on the same day with a power sprayer to bearing 8-year-old Elberta peach trees.

Lead arsenate was used at the rate of 1 pound to 50 gallons, and calcium arsenate at the rate of  $\frac{3}{4}$  pound to 50 gallons. Calcium carbonate and calcium sulphite were used at a calcium content equivalent to 4 pounds of hydrated lime to 50 gallons of spray. The results are given in Table 8.

TABLE 8.—*Spraying experiments with arsenicals, both alone and in combination with the buffer salts present in old and new dusts*

Materials used	Quantity per 10 gallons of spray	Effect on plants
	<i>Grams</i>	
Lead arsenate.....	90.8	Moderate defoliation; remaining leaves moderately burned; moderate fruit injury; no twig injury.
Lead arsenate.....	90.8	Very light defoliation; remaining leaves moderately burned; light fruit injury; no twig injury.
Hydrated lime.....	363.2	
Lead arsenate.....	90.8	Same as with hydrated lime.
Calcium sulphite.....	572.1	
Lead arsenate.....	90.8	Same as with lead arsenate alone.
Calcium carbonate.....	648.0	
Calcium arsenate.....	68.1	Almost complete defoliation; severe peach and twig injury.
Calcium arsenate.....	68.1	Moderate defoliation; moderate spotting on remaining leaves; severe fruit injury; no twig injury.
Hydrated lime.....	363.2	



A study of Table 8 leads to the following conclusions: (1) Hydrated lime greatly reduced arsenical burning in the case of both lead and calcium arsenates; (2) calcium sulphite prevented injury as well as did hydrated lime; (3) calcium carbonate was without effect in reducing burning; (4) calcium arsenate caused much more severe injury than did lead arsenate.

A 1-year-old dust of a light gray shade is therefore somewhat more likely to cause injury than a new dust; because a small part of the lead arsenate has been converted into calcium arsenate, and the remainder of the lime has been converted into calcium carbonate.

A 1-year-old dust dark gray to black is more likely to cause injury than either a new dust or a 1-year-old light gray dust, because a large portion of the lead arsenate has been converted into calcium arsenate and the remainder of the lime into calcium carbonate. The darker the dust, the more calcium arsenate is present and, therefore, the greater the danger of arsenical injury.

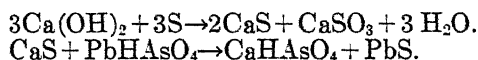
Elberta trees heavily dusted with these types of dust bore out these facts, as seen in Table 9. Frequent light rains and exceptionally high humidity made conditions especially favorable for burning. Only one application of dust was made.

TABLE 9.—*Dusting experiments with lead-arsenate-sulphur-lime mixtures*

Trees dusted with—	Effect on plants
New dust.....	No injury.
Year-old, light gray dust..	Very light leaf spotting on few leaves.
Year-old, black dust.....	Light spotting on few leaves. Very light burn on 5 per cent of the fruit.

### SUMMARY

The dark color of an old sulphur-lead-arsenate-lime dust is due to the formation of black lead sulphide during storage, the probable reactions being represented as follows:



Varying quantities of calcium arsenate are formed in different dusts, depending largely upon the extent of the above reactions. The darker the dust, the more calcium arsenate there is present.

All hydrated lime not reacting with sulphur or lead arsenate is converted into calcium carbonate within one year or less.

The results of chemical analysis indicate that little or no change in insecticidal or fungicidal value during storage should be expected.

There is some increase in danger of injuring the plant from the use of old dusts; the darker the dust, the greater the danger of injury

## LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (2) CAMPBELL, F. L.  
1926. ON THE RÔLE OF CALCIUM HYDROXIDE IN HYDRATED LIME-ACID LEAD ARSENATE SPRAYS. *Jour. Agr. Research* 32: 77-82.
- (3) CHAMOT, É. M.  
1921. ELEMENTARY CHEMICAL MICROSCOPY. Ed. 2, partly rewritten and enl., 479 p., illus. New York and London.
- (4) CHASE, W. W.  
1917. EXPERIMENTAL DUSTING AND SPRAYING OF PEACHES. *Ga. State Bd. Ent. Circ.* 21, 15 p., illus.
- (5) ———  
1918. HELPFUL HINTS ON DUSTING PEACHES. *Ga. State Bd. Ent. Circ.* 23, 7 p.
- (6) ———  
1920. EXPERIMENTAL DUSTING AND SPRAYING OF PEACHES FOR 1919. *Ga. State Bd. Ent. Circ.* 30, 13 p.
- (7) GINSBURG, J. M.  
1926. THE EFFECT OF MOISTURE, TEMPERATURE, AND LIGHT ON THE DECOMPOSITION OF LEAD-ARSENATE IN SULFUR-LIME DRY MIX SPRAY. *Jour. Econ. Ent.* 19: 841-853.
- (8) HINRICHS, C. G.  
1904. FIRST COURSE IN MICROCHEMICAL ANALYSIS. 156 p., illus. St. Louis, Mo., New York [etc.].
- (9) ROBINSON, R. H.  
1918. THE CALCIUM ARSENATES. *Oreg. Agr. Expt. Sta. Bul.* 131, 15 p.
- (10) SNAPP, O. I., TURNER, W. F., and ROBERTS, J. W.  
1922. CONTROLLING THE CURCULIO, BROWN-ROT, AND SCAB IN THE PEACH BELT OF GEORGIA. *U. S. Dept. Agr. Circ.* 216, 30 p., illus.



## RATES OF GROWTH OF IMMATURE DOUGLAS FIR AS SHOWN BY PERIODIC REMEASUREMENTS ON PERMANENT SAMPLE PLOTS<sup>1</sup>

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### SCOPE AND METHODS OF STUDY

In the region west of the Cascade Range in Washington and Oregon immature even-aged forests of pure or almost pure Douglas fir (*Pseudotsuga taxifolia*) are of common occurrence. The total area of these immature or "second-growth" forests of Douglas fir, anywhere from 1 to 120 years of age, has been estimated at 4,500,000 acres at least. They stand partly on the national forests, but a good half of them are on private lands.

These forests originated naturally, either following severe fires which killed the former forest or, more rarely, after clear cutting in a logging operation. Some that followed forest fires are very extensive, even-aged, uniformly stocked with trees, and unbroken over thousands of acres; others are but small patches surrounded by timber of another age, or occasionally are a composite of several ages.

Rapid growing as they are, these stands are of tremendous economic importance in the region. Although not now merchantable for saw logs in the general market, they will furnish an acceptable supply of stumpage for the lumber industry when the virgin supplies of large timber are gone. They now afford the forester an unexcelled opportunity to study, by either the temporary-plot or permanent-plot method, the growth and yield of Douglas fir. Such studies are of value in determining not only how fast these particular forests are growing but also what yields may be generally expected after logging and natural reforestation. Many of the stands are so uniform and well stocked that they are a fair index of what may be expected under forest management.

A yield study of these stands by the temporary-plot method was begun by the Forest Service in 1909-1911, and was continued in a more comprehensive and complete fashion and for a wider range of sites and ages in 1924-25 by the Pacific Northwest Forest Experiment Station. The results of these field studies are now being compiled for publication by R. E. McArdle. The part of this paper regarding "normality" of stands is based upon the 1924-25 study. An attempt is made here to summarize the measurements up to date, to draw any conclusions which may be definite enough, and also to present several methods of analysis of permanent sample-plot material.

<sup>1</sup> Received for publication Dec. 3, 1927; issued April, 1928.

From the beginning of these studies, permanently located sample plots were established to supplement the results obtained by the temporary plots. By 1927, 28 such permanent plots had been put in, and more are being established every year. Twelve of these plots have had two or more remeasurements.

The procedure followed in establishing the permanent sample plots was briefly as follows: Plots, preferably 1 acre in size, were laid out in typical well-stocked areas of even-aged second-growth Douglas fir. The boundaries were blazed, the corners marked by posts, and all the trees tagged by numbered metal tags at 4.5 feet above average ground level. The diameters at this point, breast height, were then measured by diameter tape or, in the earlier measurements, by calipers, and recorded by tree number. The heights of a number of trees were then taken, either by transit, Abney levels, or hypsometer, to form the basis for a height-on-diameter curve. These measurements, with additional descriptive notes, were recorded every five years, and a report on each of the groups of plots was prepared by those in charge of the project and placed on file with the Forest Service.

In order to make the results truly comparable, the whole series of measurements have been worked up under the same system. A few of the more important preliminary computations can be briefly outlined before the plot values are discussed. Anamorphic principles were used in fitting the height curves for successive periods. The most reliable set of heights was used as the basis for a graduating curve. In volume computation the diameters were grouped in single inch classes and the volumes taken from the new volume tables for second-growth Douglas fir of the Pacific Northwest, prepared for the yield study already mentioned. These tables give the total cubic foot contents inside bark of the tree; the International ( $\frac{1}{8}$ -inch kerf) board foot volume to a minimum d. b. h. (diameter at breast height) of 7 inches, a top diameter of 5 inches inside bark, and a 1.5-foot stump; and the Scribner board-foot volume to a minimum d. b. h. of 12 inches, top diameter inside bark of 8 inches, and stump height of 2 feet. No allowance for defect or waste in logging is made in any of the computations. All values are expressed in terms of the horizontal acre, although some of the plots were originally laid out by surface measure.

## DESCRIPTION OF PERMANENT SAMPLE PLOTS

### CASCADE PLOTS

In the spring of 1910 three 1-acre plots were located near Eula, Oreg., on the Cascade National Forest. (Fig. 1.) The stand was estimated to be 54 years old at that time. Recent investigation has determined the average condition of the land to be Site Quality II or, if the site-index system is used, site index 165. "Site index" is here defined as the height of the average dominant and codominant trees when the stand is 100 years of age. The basis for estimating the site index of plots younger or older than 100 years is found in curves drawn up for the Douglas fir yield study. Site-index values were computed for all periods, from which an average or most likely value was drawn. The site indices as computed showed an unexpected consistency for the several periods, any existent variability being chiefly attributable to the difference of estimates in indexing young classes.

The plots are located on a gentle north slope about 200 feet above the river valley. In this practically pure Douglas fir stand only a scattering of other species is to be found, such as madroño (*Arbutus menziesii*), golden chinquapin (*Castanopsis chrysophylla*), big-leaf maple (*Acer macrophyllum*), western hemlock (*Tsuga heterophylla*), and Pacific dogwood (*Cornus nuttallii*), all of which are in the understory. The stand is well closed and in 1910 had a crown density of

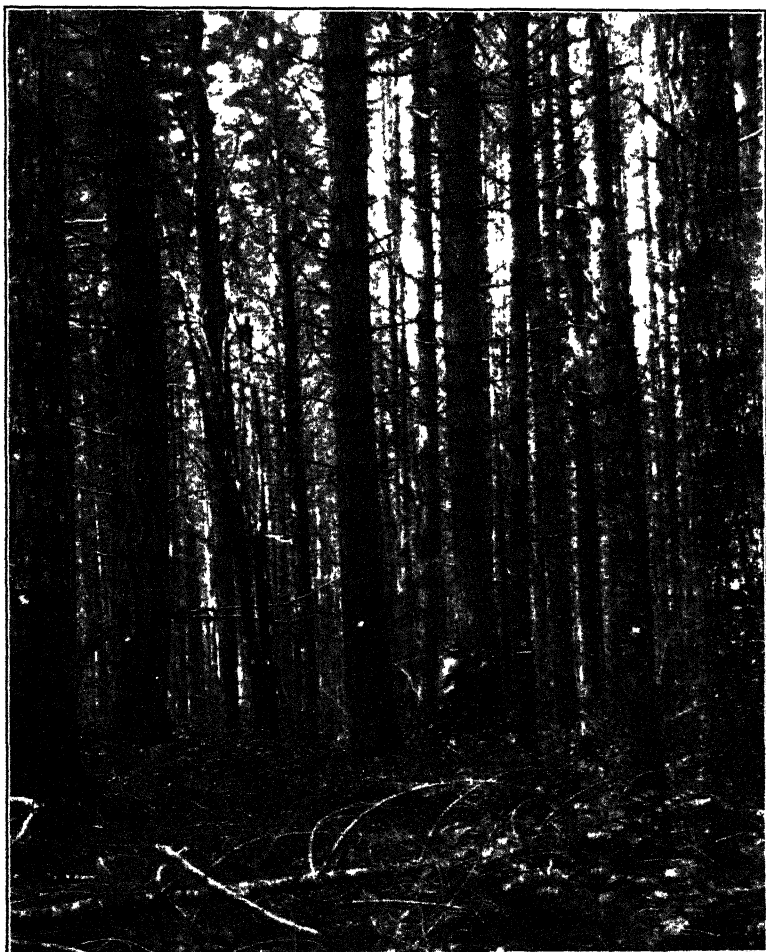


FIG. 1.—Cascade permanent sample plot No. 1, in a stand of Site Quality II, aged 69 years in 1925, volume 10,316 cubic feet, or 49,280 board feet by the Scribner rule

about 0.9. Several ice storms, notably one in 1888 and another in 1917, have produced bayonet tops and crooks and have killed some of the smaller trees. At the time of the establishment of the plots seedlings were quite numerous, but in the last measurement very few were found. Approximately three-quarters of the area is covered by underbrush, chiefly salal (*Gaultheria shallon*) and Oregon grape (*Berberis* sp.).

Since 1910 the plots have been remeasured at intervals of five years. In 1915 and 1920, however, the measurements were taken after the growth season was well advanced. For this reason the three periods instead of being taken as 5 growth years each, are assumed to be  $5\frac{1}{2}$ , 5, and  $4\frac{1}{2}$  growth years, respectively. Table 1 lists the more important plot values per acre. Only live trees are included.

TABLE 1.—Plot values per acre, permanent sample plots, Cascade National Forest \*

Year	Plot No.	Age	Number of conifer trees			Average d. b. h.	Average height	Total basal area		Volume of conifer trees		
			All	12 inches ±	7 inches ±			Conifers	Hardwoods	Cubic measure	International rule	Scribner rule
1910	1	Yrs.				In.	Ft.	Sq. ft.	Sq. ft.	Cu. ft.	Bd. ft.	Bd. ft.
	2	54	158	100	165	13.3	103	181.2	8.7	7,354	48,420	29,010
	2	54	214	124	193	13.5	104	212.5	1.6	8,706	58,770	34,710
	3	54	190	118	171	14.4	108	214.7	5.3	8,833	59,280	36,880
Average			197	114	176	13.7	105	202.8	5.2	8,298	55,490	33,367
1915	1	59½	175	106	159	14.3	116	194.2	4.0	8,709	60,270	37,440
	2	59½	198	127	189	14.6	117	231.7	8.5	10,274	71,460	44,040
	3	59½	175	124	168	15.6	121	233.2	2.2	10,406	72,820	46,410
Average			183	119	172	14.8	118	219.7	2.3	9,796	68,183	42,630
1920	1	64½	149	110	143	16.0	126	207.0	3.5	9,528	68,590	44,070
	2	64½	160	122	180	16.1	126	226.9	6	10,536	74,920	47,860
	3	64½	157	120	154	16.7	128	237.2	2.0	10,934	78,090	50,940
Average			155	117	152	16.3	127	223.7	2.0	10,333	73,867	47,623
1925	1	69	141	111	138	16.9	132	218.2	2.8	10,316	74,260	49,280
	2	69	148	123	148	17.2	132	238.7	6	11,324	80,370	53,860
	3	69	147	120	147	17.7	134	251.3	1.7	11,864	86,070	57,290
Average			145	118	144	17.3	133	236.1	1.7	11,168	80,233	53,477

\* Plots 1 and 2=1 acre, plot 3=0.95 acre; Site Quality II; site index 165, average for all periods.

In 1910 all diameters were measured with calipers. In 1915, the diameters on plots 1 and 2 were taken with calipers and on plot 3 with diameter tape; from then on the diameter tape was used exclusively. Since McArdle has found that diameter tape measurements overrun caliper measurements by approximately 1.5 per cent in basal area in stands of similar type and range of diameters, correction factors were applied to the values for the years when calipers were used. Basal areas and cubic foot volumes in Table 1 are increased by 1.5 per cent; International and Scribner board foot volumes by 2 per cent.

The periodic and mean annual increments are presented in Table 2.

TABLE 2.—Annual increments per acre, permanent sample plots, Cascade National Forest

Plot No.	Cubic measure			International rule			Scribner rule		
	First period	Second period	Third period	First period	Second period	Third period	First period	Second period	Third period
	Cu. ft.	Cu. ft.	Cu. ft.	Bd. ft.	Bd. ft.	Bd. ft.	Bd. ft.	Bd. ft.	Bd. ft.
1.....	246	164	175	2,155	1,664	1,260	1,533	1,326	1,158
2.....	285	52	175	2,307	692	1,211	1,696	764	1,333
3.....	286	106	207	2,452	1,054	1,773	1,824	906	1,411
Average.....	272	107	186	2,308	1,137	1,415	1,684	999	1,301

TABLE 2.—Annual increments per acre, permanent sample plots, Cascade National Forest—Continued

## MEAN ANNUAL INCREMENTS

Plot No	Cubic measure				International rule				Scribner rule			
	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement
	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
1.....	136	146	147	150	897	1,013	1,063	1,076	337	629	683	714
2.....	161	173	163	164	1,058	1,201	1,162	1,165	643	740	742	781
3.....	164	175	170	172	1,098	1,224	1,211	1,237	674	780	790	830
Average...	154	165	160	162	1,025	1,146	1,145	1,163	618	716	738	775

If to the volume of the live trees the volumes of trees which died during any one period were added, a truer indication of total yield up to the end of the period would be obtained. Such volume is seldom considered in young natural, unthinned stands. At times, however, it does furnish a clue to unusual increments. Thus the apparently low increments for the period 1915–1920 are explained by the extraordinary losses by storms during that period. (Table 3.)

TABLE 3.—Mortality by periods, permanent sample plots, Cascade National Forest 1911–1915

Plot No.	Number of trees	Basal area	Volume		
			Cubic measure	International rule	Scribner rule
		<i>Sq. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
1.....	13	2.81	81	258	-----
2.....	16	4.36	131	545	-----
3.....	14	2.94	83	222	-----
Average.....	14.33	3.37	98	342	-----
Average per cent ..	-----	1.33	1.00	0.50	-----

## 1916–1920

1.....	26	9.26	331	1,695	348
2.....	38	16.69	631	2,437	1,829
3.....	18	9.82	391	2,500	1,045
Average.....	27.33	11.92	451	2,211	907
Average per cent ..	-----	5.33	4.36	2.99	1.90

## 1921–1925

1.....	8	2.70	96	505	0
2.....	12	7.88	328	2,159	777
3.....	10	4.02	150	815	140
Average.....	10	4.87	191	1,160	306
Average per cent ..	-----	2.06	1.71	1.45	0.57

\* Percentages are expressed on the basis of plot values at the end of the period.

## SIUSLAW PLOTS

Four plots on the Siuslaw National Forest have also been measured four times. In 1926 three new plots were laid out with the hope of

## PLOTS 4 AND 5

In 1911 two plots were established in a 50-year-old Site Quality II stand (site indices 167 and 160) near Alpha, Oreg. Like most of the forests of this type, this one originated after a severe fire which

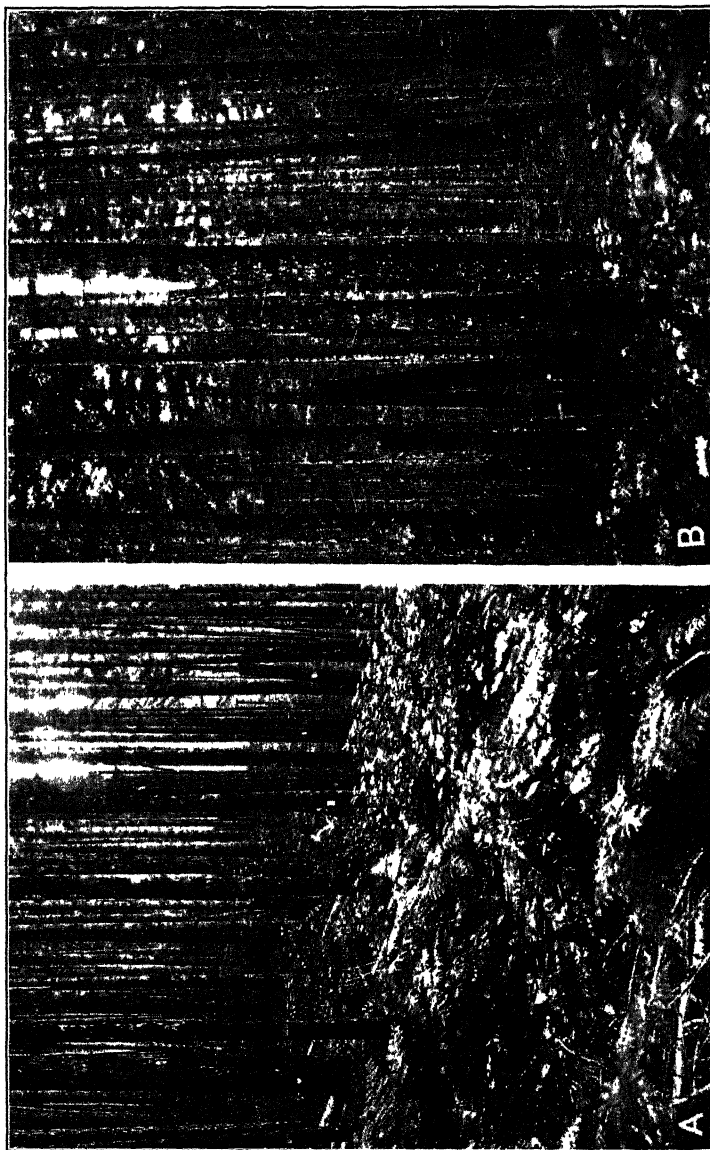


Fig. 2.—A, Permanent sample plot No. 5 on the Siuslaw National Forest, Site Quality II, aged 65 years in 1926; volume 12,397 cubic feet, or 47,880 board feet by the Scribner rule; many dead trees in stand. B, A companion plot to Siuslaw permanent sample plots Nos. 6 and 7; Site Quality I, aged 53 years in 1926, showing more even stocking than plots 6 and 7; present volume 10,984 cubic feet, or 44,400 board feet Scribner. Several stumps from the old fire which destroyed the original stand still remain.

killed practically all of the former forest. Plot 4 is on a steep 60 per cent southwest slope and plot 5 (fig. 2, A) on a moderate 20 per cent south slope. There are no rock outcrops and the soil is a deep sandy loam. Stand density was originally given as 0.85–0.9. The underbrush and ground cover are now very abundant, salal, vine



maple (*Acer circinatum*), and ferns occupying much space. The stand is in good health, although signs of a previous ice storm are numerous.

The plots were remeasured in late summer or autumn at five-year intervals. Corrections for caliper measurements were made for the 1911 values.

Mortality on plot 5 was high in the second period, resulting in a low net cubic foot increment for this period. As the mortality was confined chiefly to the smaller diameters, board foot increment was not appreciably affected.

#### PLOTS 6 AND 7

In the same year that plots 4 and 5 were laid out at Alpha, Oreg., two others were established in close proximity in a 38-year-old stand of Site I (site index 186) on Saddle Mountain, near Pawn, Oreg. Plot 6 is 1 acre and plot 7 is one-half acre in size. On each the slope and aspect are variable, since a small gully cuts through the plots. There are no rock outcrops and the soil is a deep sandy loam. The crown density of the stand was 0.75 at the time of establishment. Several holes contribute to an undesirable irregularity. The stand is in good health. A heavy underbrush and ground cover consisting chiefly of salal, together with sword fern, vine maple, and salmonberry (*Rubus spectabilis*), cover practically the whole area. Numerous old stubs, remnants of the original forest which was destroyed by fire, are found here and there.

Unfortunately the trees were not marked with metal tags until 1926. Pronounced systematic errors can arise where the trees are untagged, since the caliper man has no guide to where the diameter was last taken. A man might, for instance, take the diameters consistently low, thus increasing the diameters, the volumes, and the increments for that period. Such was evidently the case in 1921 on these two plots. Diameter, volume, and increment values are extremely erratic and must be omitted in almost all the discussions. In addition, no reliable record of mortality could be kept. In the tabulation, corrections for obtaining diameters by calipers instead of by diameter tape are made for the years 1911 and 1916. Plot values per acre and annual increments are shown in Tables 4 and 5; mortality records for plots 4 and 5 are shown in Table 6.

TABLE 4.—Plot values per acre, permanent sample plots, Siuslaw National Forest <sup>a</sup>

Year	Plot No.	Age	Number of conifer trees			Average d. b. h.	Average height	Total basal area		Volume of conifer trees		
			All	12 inches +	7 inches +			Conifers	Hard-woods	Cubic measure	International rule	Scribner rule
1911.....	4	Yrs.				<i>In.</i>	<i>Ft.</i>	<i>Sq. ft.</i>	<i>Sq. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
		50	298	185	273	13.4	102	292.8	11.9	11,980	79,290	48,350
	5	50	382	110	336	10.65	102	236.3	0	9,986	63,390	26,370
	Average.....		340	148	304	11.95	102	264.6	6.0	10,938	71,340	37,360
1916.....	6	38	199	101	181	13.1	95	187.2	3.8	7,061	44,920	25,280
		38	236	82	202	10.8	87	151.0	5.7	5,446	32,520	14,440
	7	38										
	Average.....		218	92	192	11.9	91	169.1	4.8	6,254	38,720	19,890
1916.....	4	55	273	192	263	14.9	112	329.4	9.1	14,152	96,870	61,620
		55	347	134	331	11.6	109	255.1	0	11,180	74,450	35,500
	5											
	Average.....		310	163	297	13.15	110	292.2	4.6	12,660	85,660	48,560
1921.....	6	43	183	115	177	14.45	105	208.2	3.6	8,417	56,130	33,700
		43	161	98	161	13.3	102	156.2	9.2	6,272	40,920	22,120
	7											
	Average.....		172	104	169	13.95	104	182.2	6.4	7,344	48,525	27,940
1921.....	4	60	238	190	233	16.35	120	346.6	6.7	15,520	109,000	71,870
		60	297	145	290	12.6	116	256.3	0	11,626	79,590	42,290
	5											
	Average.....		268	168	262	14.4	118	301.4	3.4	13,573	94,295	57,080
1926.....	6	48	156	131	153	17.0	119	245.6	0	10,685	74,720	49,620
		48	138	120	116	16.0	116	192.8	0	8,381	58,120	37,620
	7											
	Average.....		147	126	134	16.5	118	219.2	0	9,533	66,420	43,620
1926.....	4	65	228	192	223	17.1	125	364.1	6.5	16,888	120,450	81,510
		65	286	152	279	13.1	119	267.0	0	12,397	86,210	47,890
	5											
	Average.....		257	172	251	15.0	122	315.6	3.2	14,642	103,330	64,700
1926.....	6	53	150	131	149	17.2	127	241.9	5.9	11,223	80,110	53,880
		53	132	104	132	16.3	124	190.4	4.5	8,806	62,320	39,880
	7											
	Average.....		141	118	140	16.8	126	216.2	5.2	10,014	71,215	46,880

<sup>a</sup> Plot 4=0.399 acre, plot 5=0.455 acre, plot 6=0.932 acre, plot 7=0.441 acre. Plots 4 and 5 are Site Quality II (site indices 167 and 160), plots 6 and 7 are Site Quality I (site index 186)

TABLE 5.—Annual increments per acre, permanent sample plots, Siuslaw National Forest

## PERIODIC ANNUAL INCREMENTS

Plot No.	Cubic measure			International rule			Scribner rule		
	First period	Second period	Third period	First period	Second period	Third period	First period	Second period	Third period
4.....	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
	434	274	274	3,516	2,426	2,290	2,654	2,050	1,928
5.....	257	89	154	2,212	1,028	1,324	1,826	1,358	1,120
	Average.....	346	182	214	2,864	1,727	1,807	2,240	1,524
6.....	271	454	108	2,242	3,718	1,078	1,696	3,172	852
	165	422	85	1,680	3,440	1,536	1,536	3,100	452
7.....									
	Average.....	218	438	96	1,961	3,579	959	1,616	652

TABLE 5.—Annual increments per acre, permanent sample plots, Siuslaw National Forest—Continued

## MEAN ANNUAL INCREMENTS

Plot No	Cubic measure				International rule				Scribner rule			
	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement
4.....	<i>Cu. ft.</i> 240	<i>Cu. ft.</i> 257	<i>Cu. ft.</i> 259	<i>Cu. ft.</i> 260	<i>Bd. ft.</i> 1, 586	<i>Bd. ft.</i> 1, 761	<i>Bd. ft.</i> 1, 817	<i>Bd. ft.</i> 1, 853	<i>Bd. ft.</i> 967	<i>Bd. ft.</i> 1, 120	<i>Bd. ft.</i> 1, 198	<i>Bd. ft.</i> 1, 254
5.....	198	203	194	191	1, 268	1, 354	1, 326	1, 326	527	645	705	737
Average....	219	230	226	225	1, 427	1, 557	1, 572	1, 590	747	883	951	995
6.....	186	196	223	212	1, 182	1, 305	1, 557	1, 512	665	785	1, 034	1, 017
7.....	143	146	175	166	856	952	1, 211	1, 176	380	514	784	752
Average....	165	171	199	189	1, 019	1, 128	1, 384	1, 344	523	650	909	885

TABLE 6.—Mortality, by periods, permanent sample plots, Siuslaw National Forest

1912-1916

Plot No.	Number of trees	Basal area	Volume		
			Cubic measure	International rule	Scribner rule
4.....	25	<i>Sq. ft.</i> 7. 67	<i>Cu. ft.</i> 241	<i>Bd. ft.</i> 1, 075	<i>Bd. ft.</i> -----
5.....	37	7. 67	169	563	-----
Average.....	31	7. 67	205	819	-----
Average per cent <sup>a</sup> .....	-----	2. 62	1. 62	0. 96	-----

1917-1921

4.....	35	12. 63	418	2, 195	-----
5.....	51	16. 70	576	3, 084	-----
Average.....	43	14. 66	497	2, 640	-----
Average per cent <sup>a</sup> .....	-----	4. 86	3. 66	2. 80	-----

1922-1926

4.....	10	5. 54	214	1, 145	501
5.....	11	4. 29	163	936	-----
Average.....	10. 5	4. 92	188	1, 040	250
Average per cent <sup>a</sup> .....	-----	1. 56	1. 28	1. 01	. 39

<sup>a</sup> Percentages are expressed on the basis of plot values at the end of the period.

## COLUMBIA PLOTS

Five plots were laid out in 1914 on the Columbia National Forest in several localities and sites, and were measured successively in 1914, 1919, and 1924. Two of the plots had to be abandoned because a road was built through them in 1923. In 1924 one new plot was put in to take their place. The 1924 measurements were taken in the fall; the two previous ones were taken in the spring. The last period, therefore, contains six growing seasons.

## PLOTS 1, 2, AND 3

Along the old Race Track Trail from Hemlock Ranger Station in the Wind River Valley, Wash., three 1-acre plots were laid out in a fairly thrifty 72-year-old stand, which came in after a fire made a clean burn of the original forest. Conditions are variable and consequently the site is not the same for each plot. Plot 1 is in a Site IV stand (site index 110), and plots 2 and 3 are in Site III stands (site indices 150 and 133). Plots 1 and 2 are on gentle slopes, while plot 3 is on a steep 60 per cent slope. The soil is a deep sandy loam with some gravel and small stones. The crown density varied from 0.6 to 0.8 at the time of establishment, and the trees are uniformly distributed over the plots. The underbrush and ground cover are light and consist of scattered dogwood, western mountain ash (*Sorbus sitchensis*), vine maple, Oregon grape, fern, goatbrush (*Pachistima myrsinites*), and other species in lesser quantity.

All the plots were measured by calipers in 1914 and 1919, and plot 2, the only one remaining, by diameter tape in 1924. Corrections were applied for 1914 and 1919 to the existent plot 2 so as to bring all measurements on a comparable basis. Compared to that on some of the other plots, the death rate is rather low.

## PLOTS 4 AND 5

In a forest of similar age but on another drainage a few miles away, two more plots were established in 1914 in a stand on Site III (site index 144). Plot 4 is on a gentle to moderate west slope and plot 5 on a steep 65 per cent east slope. The soil is a loam underlain, on plot 5 at least, by boulders. Underbrush and ground cover consist chiefly of vine maple, dogwood, Oregon grape, and fern in varying densities, but never very heavy. The crown density at the time of establishment was 0.7-0.8 on plot 4 and 0.6 on plot 5. The trees are thrifty and are fairly evenly distributed. One or two small holes now present in plot 4 were caused by the death of a few trees. The mortality in this plot has been higher than in any of the other Columbia plots.

Records for the five plots are presented in Tables 7 to 9.

TABLE 7.—Plot values per acre, permanent sample plots, Columbia National Forest\*

Year	Plot No.	Age	Number of conifer trees			Average d. b. h.	Average height	Total basal area of conifers	Volume of conifer trees		
			All	12 inches +	7 inches +				Cubic measure	International rule	Scribner rule
		Yrs.				Inches	Feet	Sq. ft.	Cu. ft.	Bd. ft.	Bd. ft.
1914	1	72	251	120	224	12.1	85	200.8	6,904	40,910	21,660
	2	72	160	116	146	16.0	113	223.0	9,413	64,660	43,060
	3	72	246	146	217	13.5	99	244.1	9,393	61,910	37,920
	4	72	185	127	177	14.7	110	218.1	9,089	61,130	38,160
	5	72	157	127	150	16.6	112	236.0	9,852	67,820	44,980
1919	1	77	243	130	220	12.8	94	214.9	7,888	48,930	26,770
	2	77	149	117	141	17.2	124	239.8	10,786	76,820	51,410
	3	77	241	159	213	14.5	109	276.3	11,354	76,350	48,500
	4	77	176	133	170	15.6	113	234.6	10,201	71,090	45,570
	5	77	154	130	147	17.5	122	257.3	11,425	81,120	55,020
1924	1										
	2	83	145	121	139	18.0	131	256.1	11,953	86,290	58,850
	3										
	4	83	171	133	166	16.4	126	249.8	11,348	80,510	52,440
	5	83	151	132	148	18.3	130	276.2	12,862	92,900	64,050

\* Plot 1=1 acre; plot 2=0.995 acre; plot 3=0.936 acre; plot 4=0.975 acre; plot 5=0.938 acre. Plot 1=Site Quality IV (site index 110); plots 2 to 5=Site Quality III (site indices 150, 133, 144, and 144, respectively).

TABLE 8.—Annual increments per acre, permanent sample plots, Columbia National Forest

## PERIODIC ANNUAL INCREMENTS

Plot No.	Cubic measure		International rule		Scribner rule	
	First period	Second period	First period	Second period	First period	Second period
	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
1.....	197		1,604		1,022	
2.....	275	194	2,432	1,578	1,670	1,240
3.....	392		2,888		2,116	
4.....	222	191	1,992	1,570	1,482	1,145
5.....	315	240	2,660	1,963	2,008	1,505

## MEAN ANNUAL INCREMENTS

Plot No.	Cubic measure			International rule			Scribner rule		
	First measurement	Second measurement	Third measurement	First measurement	Second measurement	Third measurement	First measurement	Second measurement	Third measurement
	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
1.....	96	102		508	635		301	348	
2.....	131	140	144	898	998	1,040	598	608	709
3.....	130	147		860	992		527	630	
4.....	126	132	137	849	923	970	530	592	632
5.....	137	148	155	942	1,054	1,119	625	715	722

TABLE 9.—Mortality by periods, permanent sample plots, Columbia National Forest

1915-1919

Plot No.	Number of trees	Basal area	Volume		
			Cubic measure	International rule	Scribner rule
			<i>Cu. ft.</i>	<i>Bd. ft.</i>	<i>Bd. ft.</i>
1.....	8	<i>Sq. ft.</i> 1.32	30	73	
2.....	11	2.80	73	283	
3.....	4	1.62	46	226	
4.....	8	4.78	177	1,075	500
5.....	3	1.80	57	320	107
Average per cent <sup>a</sup> .....		1.01	0.75	0.56	0.27

1920-1924

2.....	4	1.86	59	302	
4.....	5	2.62	100	622	303
5.....	2	1.17	38	212	
Average per cent <sup>a</sup> .....		0.72	0.54	0.44	0.17

<sup>a</sup> Percentages are expressed on the basis of plot values at the end of the period.

## DISCUSSION OF PLOT VALUES

## VOLUMES

The total yields up to the time of last measurement, an important point of comparison, if arranged by site classes and rounded off to the nearest thousand can be quickly summarized. The Site I plots (Siuslaw 6 and 7) present age 53 trees have average

cubic foot, international board foot, and Scribner board foot of 10,000, 71,000, and 47,000, respectively. Three of the Site II plots (Cascade 1, 2, 3), age at last measurement 69 years, have average values of 11,000, 80,000 and 53,000 for these three standards of measurement, while the other two Site II plots (Siuslaw 4 and 5), age 65 years, carry 15,000, 103,000, and 65,000 feet. Disproportionately large values are caused by the inclusion of plot 4. All the way through, this plot has appeared erratically high. This is due partly to the fact that the plot was laid out by surface measure, and the steep slope upon which it lies necessitates a large area correction, increasing thereby all values on the plot.

Furthermore, the upper edge of the plot is along a ridge upon which is a strip of trees of large diameter. It takes only a few of such diameters to increase the volumes tremendously. If plot 5, which is more uniform than plot 4, is considered by itself the agreement with the Cascade plots is pronounced. The Site III plots which still exist (Columbia 2, 4, 5), age 83 years at last measurement, have an average cubic foot volume of approximately 12,000 feet, International board foot volume of 87,000 feet, and Scribner board foot volume of 58,000 feet. The relation of these values to the volumes as determined in the yield study for second-growth Douglas fir will be discussed later.

#### INCREMENTS

An inspection of the various periodic increment tables serves to show how very unstable such values are. Any unusual amount of mortality is reflected immediately, especially in the cubic foot volume increments which are so largely made up from the smaller trees having no appreciable board-foot volumes but significant cubic foot volumes. Naturally the mean annual increments run much more regularly. The mean annual increments of the Site III plots (Columbia) with the present age of 83 years are still increasing in all volumes; those of the Site II plots (Cascade 1, 2, and 3, Siuslaw 4 and 5, omitting 6 and 7), present ages 69 and 65 years, respectively, have practically if not certainly reached their maximum in cubic foot and International board-foot volume increments but are still increasing significantly in Scribner volume increments; those of the young Site I stands (now 53 years old) have not reached their peak as yet. However, for all the plots, with the exception of the doubtful plots (Siuslaw 6 and 7), the periodic annual increments are on the whole decreasing. Mortality during the middle period on the Cascade plots disguises the effect, but does not leave much doubt, especially if the diameter growth of the individual diameter classes is observed.

#### BOARD FOOT-CUBIC FOOT RATIOS

Within certain limits board foot-cubic foot ratios increase steadily with age, as is apparent in the tabulation of ratios. (Table 10.) The International board foot-cubic foot ratios are still increasing slowly, but have reached a position where further increase will be very small, especially in the older plots. Scribner ratios, however, continue to enlarge at a somewhat faster rate, and undoubtedly will continue to do so until the 12-inch diameter has been reached by all trees, this diameter being the minimum for which Scribner volumes were computed, as compared to 7 inches for International volumes.

TABLE 10.—Board foot-cubic foot ratios

Plot No.	International rule				Scribner rule			
	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement
Cascade.								
1.....	6.58	6.92	7.20	7.20	3.94	4.30	4.63	4.78
2.....	6.75	6.96	7.11	7.10	3.99	4.29	4.54	4.76
3.....	6.71	7.00	7.14	7.25	4.12	4.46	4.66	4.83
Columbia.								
1.....	5.92	6.20	-----	-----	3.14	3.39	-----	-----
2.....	6.87	7.12	7.22	-----	4.57	4.77	4.92	-----
3.....	6.59	6.72	-----	-----	4.04	4.27	-----	-----
4.....	6.73	6.97	7.09	-----	4.20	4.47	4.62	-----
5.....	6.88	7.10	7.22	-----	4.56	4.82	4.98	-----
Suslaw								
4.....	6.62	6.84	7.02	7.13	4.04	4.35	4.63	4.83
5.....	6.40	6.66	6.85	6.95	2.66	3.18	3.64	3.86
6.....	6.36	6.67	7.69	7.14	3.58	4.01	4.64	4.80
7.....	5.97	6.52	6.93	7.08	2.65	3.53	4.49	4.53

## RELATION OF PERMANENT SAMPLE-PLOT DATA TO YIELD TABLES

The degree of normality of these plots or the relation of the plot values to values given in the normal yield tables for second-growth Douglas fir leads to a number of conclusions which may prove valuable in the application of such tables, or at least suggest the nature of the results which can be obtained from a more detailed study. (Table 11.)

TABLE 11.—Relation of permanent sample-plot data to normal yield tables

[Values from sample-plot data are shown in percentage of normal yield for the four successive measurements]

Plot No.	Number of trees				Basal area				Volume by International rule				Volume by cubic measure				Volume by Scribner rule			
	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement	First measurement	Second measurement	Third measurement	Fourth measurement
Cascade:																				
1.....	66	72	72	70	80	83	84	85	81	89	98	88	78	83	92	84	94	97	97	94
2.....	76	81	73	73	94	98	92	93	98	115	96	96	92	98	92	93	113	111	105	103
3.....	68	72	72	72	96	98	96	98	99	108	101	102	94	99	97	98	118	120	112	109
Columbia:																				
1.....	64	68	-----	-----	94	98	-----	-----	106	114	-----	-----	98	106	-----	-----	145	144	-----	-----
2.....	68	70	74	-----	88	92	97	-----	84	93	96	-----	83	90	94	-----	96	100	101	-----
3.....	86	92	-----	-----	102	111	-----	-----	100	114	-----	-----	97	110	-----	-----	118	131	-----	-----
4.....	72	77	80	-----	88	91	94	-----	85	94	96	-----	84	90	94	-----	94	100	100	-----
5.....	63	68	71	-----	94	100	104	-----	94	105	110	-----	92	100	106	-----	112	120	122	-----
Suslaw.																				
4.....	98	102	101	105	137	140	144	146	149	156	154	154	130	146	148	146	188	184	180	171
5.....	113	118	113	120	110	112	108	108	127	128	120	116	119	120	114	112	122	122	118	111
6.....	62	66	66	70	100	103	135	110	122	117	128	115	100	107	118	109	169	144	158	130
7.....	73	60	59	62	81	77	89	83	88	85	100	90	76	80	92	86	96	94	116	96

In the yield study for Douglas fir, "normality" is not defined as the maximum volume that may be produced by intensive care and treatment of stands, but as the volume produced under conditions of average full stocking of stands which remain unthinned throughout their lives. The conception of "normality" of the stand and the most appropriate index of "normality" raise questions which have

been debated frequently. Some identifying character or index is needed whereby the normality of the stand may be easily recognized. Several tests have been made to determine the best index of normality; such an index as might be employed when yield tables are to be applied to stands or portions of stands which are other than 100 per cent stocked or in full conformity to yield-table values. For this purpose total number of trees per acre has at times been used with other species in other regions.

Douglas fir percentages of normal number of trees did not conform at all to the percentages of normal volumes as indicated by the permanent sample plots. On the other hand, the relation of percentages of normal basal area and the percentages of normal volumes (see fig. 3) took trends similar to those obtained with number of trees in a yield study which the writer conducted in red spruce stands

of the northeastern United States.

If a graph similar to Figure 3 be drawn with number of trees as index of normality, and using values in Table 11, several interesting points are brought out. An irregular distribution of points results in a large scatter for the lower percentages (number of trees) and abnormal relations in the upper percentages. If the plots conformed strictly to the yield tables, without considering the average or standard deviations of the table values, one would expect that when num-

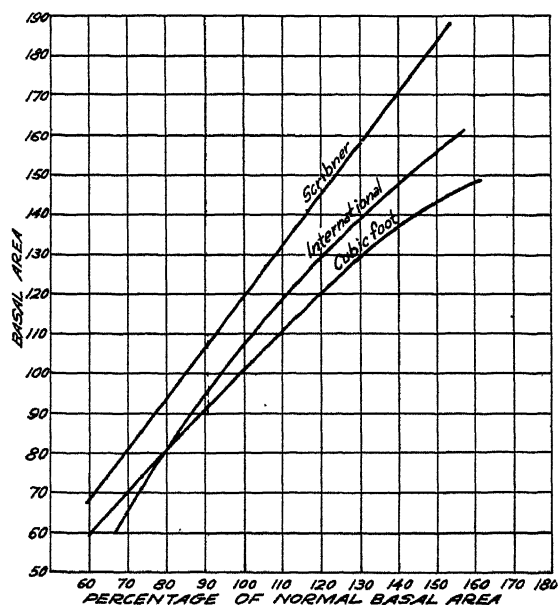


FIG. 3.—Relation of plots to normal yield tables

ber of trees is 100 per cent the curves would pass through 100 per cent basal area and 100 per cent volume. Such is not the case, for, at 100 per cent number of trees, the percentages, especially those for the Scribner volume, run considerably higher. The extreme scatter of the points does not permit the placing of a satisfactory curve.

Indirectly this lack of coordination in normality percentages is indicative of variability in diameter distribution and spacing of trees in a plot, which the most careful selection of plots seldom overcomes.

If now the trend of volume percentages on basal area percentages is studied, an entirely different situation obtains. (Fig. 3.) The relation between cubic foot percentage and basal area percentage is extremely regular. Between International board-foot volume and basal area it is less regular but is still well defined. The 100-100 per cent is closely approached by the curved cubic-foot volume percentages, while International volume passes within a +9 per cent. Both in-



crease regularly in roughly parallel trends with the increase in basal area. The standard errors of the curves are  $\pm 4.53$  per cent for the cubic foot-basal area relation and  $\pm 6.18$  per cent for the International-basal area relation. Scribner-basal area relation is too weak ( $SE = \pm 13.66$  per cent) to be of much value.

The curves in Figure 3 are based not only on data drawn from the 12 plots which have had at least two measurements, but also on data of all plots established previous to 1927, totaling 20 in number.

So far the relationships have been treated only as static measurements. It remains to determine whether they are static throughout the life of the stands or whether there is a fast or slow advance to normality of stocking. A simple way to observe this trend is to divide the stands into groups of normality percentages and to obtain average percentage rise or fall in stocking with each five-year period. The measurements of Siuslaw plots 6 and 7 and of the second period of Cascade plot 2 are omitted, leaving 22 periods as a working basis. This procedure is justifiable since some of the values are known to be erratic and not representative of normal growth.

From Table 12 approach towards normality is evident. Below 100 per cent stocking in any of the factors the trend is up to 100 per cent, but above 100 per cent stocking it is irregular, sometimes up and sometimes down. For instance, if an acre is below 100 per cent in basal area, in five years it will stand 2.9 per cent higher in the scale of normality; if deficient in International board-foot volume, the increase in normality percentage in five years will be 6.1 per cent. An increased number of period measurements is necessary before such values can be more certainly defined and curvilinear relations assigned.

TABLE 12.—Approach to normality expressed in percentage increase for each five-year period

Percentage of normality at beginning of period	Number of trees		Cubic measure		Basal area		Volume			
							International rule		Scribner rule	
	Num- ber- peri- ods	Per- cent- age rise or fall	Num- ber- peri- ods	Per- cent- age rise or fall	Num- ber- peri- ods	Per- cent- age rise or fall	Num- ber- peri- ods	Per- cent- age rise or fall	Num- ber- peri- ods	Per- cent- age rise or fall
	1	2	1	2	1	2	1	2	1	2
60-69	6	+4.0								
70-79	9	+1.7								
80-89	1	+6.0	1	+5.0						
90-99	1	+4.0	4	+3.5	5	+2.4	5	+5.0		
100-109	2	+1.5	10	+4.8	9	+2.8	6	+7.0	5	+2.0
110-119	3	+2.3	1	+6.0	3	+4.3	5	+4.2	3	-0.3
120-129			2	-0.5	2	-1.0			6	+2.3
130-139			1	-6.0			3	-3.7	4	-2.5
140-149			1	+16.0	1	+3.0				
150-159			2	0.0	2	+3.0	1	+7.0	1	-1.0
160-169							2	-1.0		
180-189									3	-5.6
	22		22		22		22		22	
Average below 100 per cent		+2.9		+4.5		+2.7		+6.1		+2.0
Average above 100 per cent		+2.0		+2.1		+2.5		+1.4		-0.9
Total average		+2.7		+3.7		+2.6		+3.7		-0.2

This change in position relative to the normal yield tables affects directly the increments, especially the periodic annual increments. A larger periodic increment can be expected on the understocked plots than is indicated by the yield tables. For instance, the ages and the site represented in the Cascade plots should have approximately periodic annual cubic foot volume increments ranging between 210 and 170 cubic feet, decreasing for 15 years, while actually they average 185 cubic feet per year for the 15 years in spite of the great loss during the middle five-year period.

#### AVERAGE DIAMETERS AND DIAMETER GROWTH

The average diameters of the plot groups indicate rapid growth. The Site I Siuslaw plots 6 and 7 increased from 11.9 to 16.8 inches, an average of approximately 1 inch every 3 years. The Site II Cas-

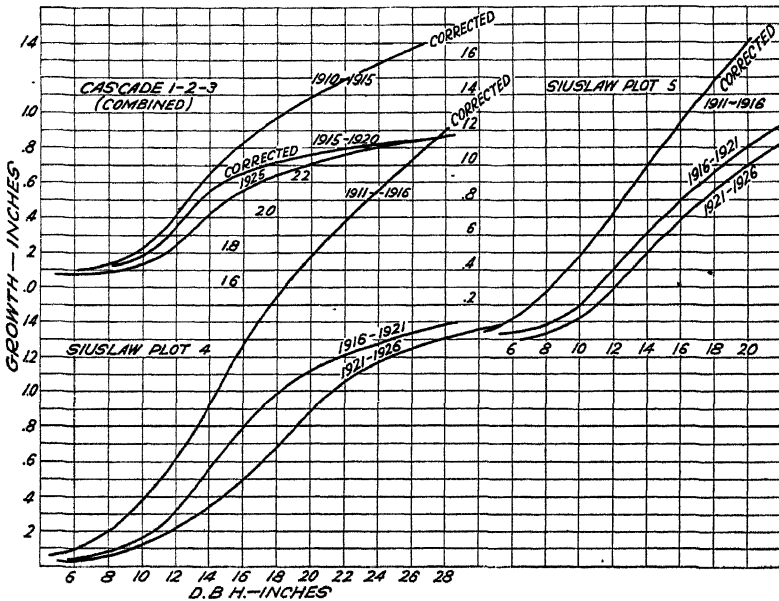


FIG. 4.—Diameter growth by inch classes, five-year periods

cade plots as an average increased from 13.7 inches to 17.3 inches in 15 years, which amounts to more than 1 inch every 5 years. The Site II Siuslaw 4 and 5 plots increased from 11.95 to 15 inches, or approximately 1 inch every 5 years. Site III Columbia plots 4 and 5 grew from 15.6 to 17.4 and plot 2 from 16 to 18 inches in 11 years, or an average of 1 inch every 6 years.

The rate of change of the average diameter of the plot is, however, no index of the rate of change of the diameters of the individual trees. From the original tallies of a number of the plots, the actual diameter growth was recorded and averaged for each period by diameter classes. (Fig. 4.) The diameter increment in all the diameter classes is steadily decreasing. This can usually be expected in stands which are left untreated by artificial thinning and subjected only to natural thinning.

Siuslaw plot 4 has been used in this series of graphs to illustrate definitely how the inclusion of exceptional diameter growth increases the periodic annual increments of the first period. An allowance for caliper readings which was made before the values were plotted has not resulted in making a much more normal diameter relation for the first period. Siuslaw plot 5 effects a similar but less pronounced distortion.

#### DISTRIBUTION OF TREES IN DIAMETER CLASSES

The distribution of diameters in a stand is thought to follow certain definite laws. Whether this distribution is in strict accordance with the law of probabilities or whether there exists some tendency to asymmetry or skewness is a question to be solved for each

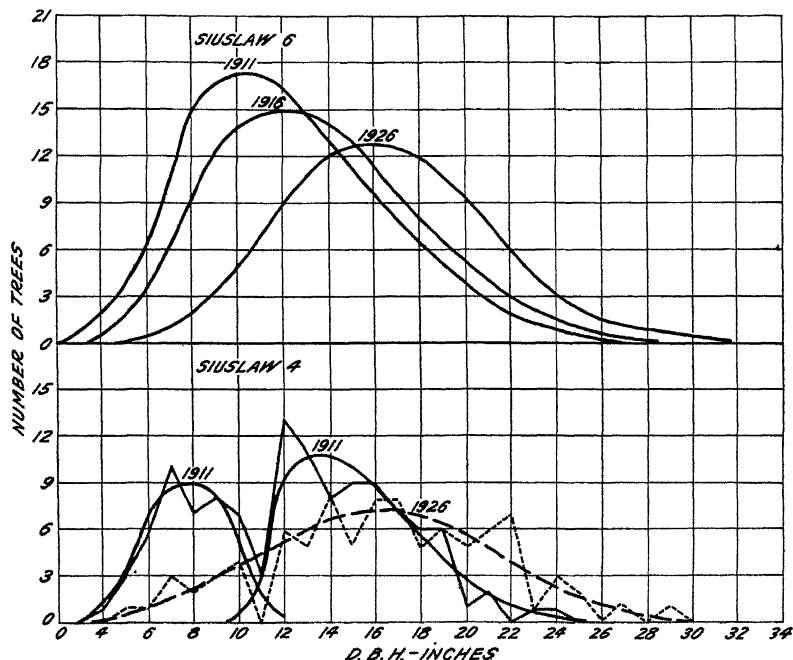


FIG. 5.—Distribution of diameters in stands

individual species. It seems natural to presume that the more tolerant a species is, the longer will the smaller diameters hold on to life and the more skewed to these diameters will the distribution be. Also the younger a plot is, the more will the distribution of trees in diameter classes be skewed to the smaller diameters. Such a situation can be shown graphically, as in the frequency curve (fig. 5) of Siuslaw plot 6 at ages 38, 43, and 53. Age 48 has been left out as being undeniably erratic. The curves shown in the graph were obtained by drawing a smooth curve through a histogram illustrating the number of trees in each diameter class plotted upon the diameter as abscissa. They fit the distribution as closely as can be judged ocularly. The number of trees is the actual number on the plot and not the number per acre. It is obvious even from such a rough graph that there is a change in the character of diameter distribution with the

age of the stand. Similar effects are obtained on all plots. The first and last stages of diameter distribution shown for Siuslaw plot 4 indicate a unique case. Apparently there are two distinct frequencies at the beginning which gradually lose their distinction with age and are finally combined into one fairly normal curve. This double frequency is not due to difference in age but to the very apparent difference of spacing, which affects the diameter development within the plot, as previously mentioned. If Columbia 2 were plotted, an asymmetry of opposite nature to Siuslaw 4 would be found. The question now arises how such distributions can be more accurately defined.

The following method has been adapted from Charlier's method of computation for skewed frequencies made use of by Y. Ilvessalo in a Finnish publication.<sup>2</sup> Of all theoretical fittings of distribution, it seems about the most easily applicable. In essence, the method consists in computing from each distribution two coefficients (coefficient of asymmetry,  $B_3$ , and coefficient of excess,  $B_4$ ) which, when applied to a definite formula and to a set of tables known as Charlier's tables, will result in giving a theoretically fitted distribution. With a large number of plots, the plots could be grouped by desirable classes and a stand table made which would then be subjected to the first part of the treatment, namely, through the computation of the coefficients up to the application of Charlier's tables. These coefficients could then be averaged or plotted in any desired manner, such as plotting on age, or averaging by type, and the curved or averaged coefficient values could be used for obtaining a correct theoretical stand table.

The scheme of computation is shown applied to Siuslaw plot 5, 1916 (area 0.455 acre).

D. b. h.	Class (c)	Number of trees (NT)	c(NT)	c <sup>2</sup> (NT)	c <sup>3</sup> (NT)	c <sup>4</sup> (NT)
5	-6	1	-6	36	-216	1,296
6	-5	6	-30	150	-750	3,750
7	-4	16	-64	256	-1,024	4,096
8	-3	24	-72	216	-648	1,944
9	-2	19	-38	76	-152	304
10	-1	16	-16	16	-16	16
11	0	15	0	0	0	0
12	+1	13	13	13	13	13
13	2	9	18	36	72	144
14	3	8	24	72	216	648
15	4	11	44	176	704	2,816
16	5	7	35	175	875	4,375
17	6	3	18	108	648	3,888
18	7	5	35	245	1,715	12,005
19	8	2	16	128	1,024	8,192
20	9	1	9	81	729	6,561
21	10	1	10	100	1,000	10,000
22	11	1	11	121	1,331	14,641
Sums = 8		158	-226 +233 +7	+2,005	-2,806 +8,327 +5,521	+74,689

The first step in the computation is to list the number of trees by diameter classes, then to give to the diameter group that is estimated

<sup>2</sup> ILVESSALO, Y. TUTKIMUKSIA METSÄTYYPPIEN TARKASTOORISESTA MERKITYKSESTÄ. [INVESTIGATIONS ON THE IMPORTANCE OF FOREST TYPES IN FOREST MENSURATION AND VALUATION.] Acta Forest. Fennica 15, 157 p., illus. 1920. (Separately paged.) [German résumé, 26 p.]

to be near the average value the class value of zero and to all other diameter classes progressive plus or minus values, as shown in the illustration. The fourth column is obtained by multiplying the class value by the number of trees in that class; the fifth column is obtained by multiplying the square of the class value by the number of trees in the class, and so on for the remaining columns. The columns are summed up with strict consideration of the plus and minus signs. Following this, a second set of computations is made.

$$d_1 = \frac{\text{Sum } c(NT)}{\text{Sum } (NT)} = \frac{+7}{158} = .044$$

$$d_2 = \frac{\text{Sum } c^2(NT)}{\text{Sum } (NT)} = +12.690$$

$$d_3 = \frac{\text{Sum } c^3(NT)}{\text{Sum } (NT)} = 34.943$$

$$d_4 = \frac{\text{Sum } c^4(NT)}{\text{Sum } (NT)} = 472.715$$

$$d_1^2 = +.002$$

$$d_1^3 = +.000$$

$$d_1^4 = +.000$$

$$\text{Average DBH} = 11 + d_1 = 11.044$$

Standard deviation squared =

$$SD_1^2 = d_2 - d_1^2 = 12.690 - .002 = 12.688$$

$$SD_1 = 3.562$$

$$SD_1^3 = 45.195$$

$$SD_1^4 = 160.985$$

Total basal area of plot (square feet)

$$= (NT) \times \frac{.7854 (\text{av. DBH}^2 + SD_1^2)}{144}$$

$$= 158 \times \frac{.7854 (134.658)}{144} = 116.04 \text{ square feet}$$

The above shows first the computation of several deviation values ( $d_1, d_2, d_3, d_4$ ), arrived at by dividing the fourth, fifth, sixth, and seventh columns of the first tabulation by the total number of trees. The actual average diameter of the stand is found by adding to the assumed average diameter the value of  $d_1$ , taking the sign into consideration. The square of the standard deviation ( $SD$ ) is obtained by subtracting from  $d_2$  the square of  $d_1$ . The total basal area of the stand is found by multiplying the area of a circle the square of whose diameter is equal to the square of the average diameter of the stand, plus the square of the standard deviation, by the total number of trees. This method of computing total basal area checks out very

closely with the value obtained in the ordinary way. A further calculation must now be made.

Check		
$d_3 = +34.943$	$d_4 = 472.715$	$SD_4 = +466.417$
$-3d_1d_2 = -1.675$	$-4d_1d_3 = -6.150$	$4d_1SD_3 = +5.855$
$2d_1^3 = .000$	$6d_1^2d_2 = +.152$	$6d_1^2SD_1^2 = +.152$
	$-3d_1^4 = .000$	$d_1^4 = .000$
<hr/>	<hr/>	<hr/>
$SD_3 = +33.268$	$SD_4 = 466.717$	472.724 should check with $d_4 = 472.715$

$$\frac{SD_3}{SD_1^3} = \frac{33.268}{45.195} = +.736 \quad \frac{SD_4}{SD_1^4} - 3 = \frac{466.717}{160.985} - 3 = 2.899 - 3 = -.101$$

$$\text{Coefficient of asymmetry} = B_3 = \frac{.736}{-6} = -.123$$

$$\text{Coefficient of excess} = B_4 = \frac{-.101}{24} = -.0042$$

The several combinations of the individual "d" values lead finally to quantities which give directly the coefficients of asymmetry and excess mentioned previously. A check computation is also included which indicates whether substantial errors were made in all previous computations.

The standard errors of the computed quantities are found:

$$\text{Error of average DBH} = \frac{SD}{\sqrt{NT}} = \frac{3.562}{\sqrt{158}} = \pm .283$$

$$SD = \frac{SD}{\sqrt{2NT}} = \frac{3.562}{\sqrt{316}} = \pm .200$$

$$B_3 = \frac{1.9325}{3\sqrt{NT}} = \frac{1.9325}{3\sqrt{158}} = \pm .051$$

$$B_4 = \frac{.6124}{3\sqrt{NT}} = \frac{.6124}{3\sqrt{158}} = \pm .016$$

A word or two in explanation before proceeding further. The first of these two coefficients ( $B_3$ ) defines the amount and character of the skewness, that is to say, the extent a distribution is warped from the normal and the direction in which it is warped. The second coefficient ( $B_4$ ) defines to what extent a given distribution exceeds the normal at both the center and the ends of the distribution. If  $B_3$  and  $B_4$  equal 0 the distribution is normal. A minus  $B_3$  indicates skewness toward the small diameters, a plus  $B_3$  one toward the large diameters. The larger the coefficient is the more pronounced is the asymmetry. A positive  $B_4$  indicates that the frequency exceeds the normal near the mean and at the two ends of the distribution and is less than the normal in the region between these portions of the curve. A negative  $B_4$  indicates the opposite condition.

\* The formula of theoretical frequencies into which these coefficients are substituted and the solution of which is brought about by Charlier's tables is:

$$F(x) = \frac{NT}{SD}(\phi_{0(x)} + B_3\phi_{3(x)} + B_4\phi_{4(x)})$$

and the computation follows this tabulated form:

Computation of theoretical frequencies

$x_1$	$x_2$	$x_3$	$x_4$	
$\frac{x_1 - \text{Av. DBH}}{SD}$	$\frac{x_2 - \text{Av. DBH}}{SD}$	$\frac{x_3 - \text{Av. DBH}}{SD}$	$\frac{x_4 - \text{Av. DBH}}{SD}$	
$\phi_{0(1)} =$ $B_3\phi_{3(1)} =$ $B_4\phi_{4(1)} =$	For diameter $x_1$ in terms of Av. DBH and SD as above from Charlier's tables		$\phi_{0(1)} =$ $B_3\phi_{3(1)} =$ $B_4\phi_{4(1)} =$	
Sum <sub>1</sub> =			Sum <sub>4</sub>	
$\frac{NT}{SD}$ Sum <sub>1</sub> =	$\frac{NT}{SD}$ Sum <sub>2</sub> =	$\frac{NT}{SD}$ Sum <sub>3</sub> =	$\frac{NT}{SD}$ Sum <sub>4</sub> =	
The results are the theoretically fitted values.				

The first step in this stage is to present the difference between each diameter group ( $x_1, x_2$ , etc.,) and the average diameter in terms of the standard deviation ( $\frac{x - \text{Av. DBH}}{SD}$ ). Then from Charlier's tables with  $B_3, B_4$  and the above known, the function values  $\phi_{0(x)}, B_3\phi_{3(x)}, B_4\phi_{4(x)}$  can be read off for each diameter. These three function values are summed, multiplied by the total number of trees, and divided by the standard deviation. This gives the number of trees to be found theoretically in each diameter group.

This last stage is not applied to the plots represented in this study, since there are too few values to form a basis for reliable stand tables. However, a number of  $B_3$  and  $B_4$  values were computed for representative plots, and are listed to show the trends of these coefficients and hence of the diameter distribution of these plots. (Table 13.)

TABLE 13.—Coefficients of asymmetry and excess

Pilot	$B_3$				$B_4$			
	First	Second	Third	Fourth	First	Second	Third	Fourth
Siuslaw 4.....	-.023	-.020	+.003	+.005	-.030	-.026	-.019	-.017
Siuslaw 5.....	-.125	-.123	-.123	-.107	+.002	-.0042	+.010	+.004
Columbia 2.....	+0.54	+0.065	+0.074	.....	-.001	-.001	-.001	.....
Cascade 1.....	-.037	-.027	+.006	+.023	-.035	-.033	-.028	-.028

All four plots illustrate the tendency to proceed in their distribution of diameters from a negative skewness (an asymmetry toward the small diameters) on to normal form and beyond into a more positive skewness (an asymmetry toward the large diameters). The addition of more plots to form composite stand tables would make the advance more definite and would decrease the standard errors,

which have proved large for plots with so few trees and as a result have made the values of the coefficients uncertain. A coefficient value greater than three times its standard error is usually taken as a significant value.

#### SUMMARY

The outstanding facts brought out so far in this study of a few permanent sample plots through a period of 15 years may be briefly summarized as follows:

**REGION.**—Even-aged stands of pure second-growth Douglas fir are of common occurrence in Oregon and Washington west of the Cascade range.

**PLOTS.**—In this region in the last 15 years at various times a total of 20 permanent sample plots have been established upon which periodic growth measurements have been made. Of these, 12 have been measured more than once and the results analyzed. Three plots on the Cascade National Forest are in a Site II forest, present age 69 years, and have been under observation for 15 years. Two plots on the Siuslaw Forest are in a Site II stand, now 65 years old, and have also been under observation for 15 years. Two others on the same forest, remeasured for an equal length of time, are in a Site I stand, now 53 years old. On the Columbia five plots in an 83-year-old stand, one on Site IV and four on Site III, have been observed for 10 years.

**VOLUME.**—At the last measurement the Cascade plots averaged a volume per acre of approximately 11,000 cubic feet, 80,000 board feet International rule, or 53,000 board feet Scribner rule; the three existent Columbia plots average now 12,000 cubic feet, 87,000 board feet International, or 58,000 board feet Scribner; the Site II Siuslaw plots (65 years old) carry per acre 14,600 cubic feet, 103,000 or 64,000 board feet International and Scribner, respectively, and the Site I Siuslaw plots (53 years old) 10,000 cubic feet, 71,000 board feet International, or 47,000 board feet Scribner.

**MORTALITY.**—On an average, death takes about 2 per cent of the basal area every five years, or about 2 per cent of the cubic foot volume. Since the dead trees are chiefly of the smaller diameter this means about 1 per cent of the International board-foot volume and less than 0.5 per cent of the Scribner volume.

**INCREMENTS.**—The mean annual increments for cubic feet and International board feet have all neared or passed their maximum, but for Scribner volume they are still approaching it in all cases.

**BOARD FOOT-CUBIC FOOT RATIOS.**—Board foot-cubic foot ratios which show the average number of board feet to the cubic foot, computed by dividing the total board-foot volume of a plot by the total cubic-foot volume, are known to increase normally with increase in age. In these plots, the International ratio seems to have reached its high point. Practically all trees have passed the minimum diameter of 7 inches, and further increase can be expected to be much slower than in the early life of the stand. The Scribner ratio, since it is based upon a minimum diameter of 12 inches, still shows a decided increase, for a good proportion of the trees lie below the 12-inch minimum.

**NORMALITY.**—Based upon the relation of the sample-plot values to the values shown in the tables of the recently completed yield study for second-growth Douglas fir, expressed in percentages of the



first to the second, called "normality" percentages, tentative conclusions are drawn as to the approach of abnormally stocked stands to a normally stocked condition. The amount of change in normality percentage for a five-year period is different for each factor. For plots below 100 per cent the average advance in normality for a five-year period for total basal area is 2.7 per cent, for cubic foot 4.5 per cent, for International volume 6.1 per cent, for Scribner volume 2 per cent.

**DIAMETER GROWTH.**—On Site I average diameters increase at the rate of 1 inch in 3 years during the ages covered by these permanent plot studies, on Site II 1 inch in 5 years, on Site III 1 inch in 6 years, while on the one Site IV it takes almost 7 years for the average diameter to increase 1 inch. The diameter growth per tree increases on an average with the size of the tree and is shown graphically for several cases. Every period shows a decrease in diameter increment in each diameter class from the preceding period.

**NUMBER OF TREES IN EACH DIAMETER CLASS.**—The character of the curve of stem distribution in diameter classes evidently follows definite trends as the stand grows older, such as from a skewness toward small diameters to a more normal or symmetrical distribution and beyond that to a more and more positive skewness (an asymmetry to the large diameters). Double frequencies or distributions with two peaks seem to merge into one in the course of time.



# A ROT OF GLADIOLUS CORMS CAUSED BY *PENICILLIUM GLADIOLI*, L. McC. AND THOM<sup>1</sup>

By LUCIA McCULLOCH, *Associate Pathologist, Pathological Laboratory, Bureau of Plant Industry*, and CHARLES THOM, *Senior Mycologist, Bureau of Chemistry and Soils, United States Department of Agriculture*

## INTRODUCTION

In May, 1926, a large shipment of gladiolus corms of the Odin variety arriving in the United States from the Netherlands was held up by the plant quarantine inspection service because of the generally bad condition of the corms. A casual examination showed that many were extensively rotted. Various fungi were fruiting on the surface, and mites were present. In an effort to discover what organism was mainly responsible for the trouble, examinations and cultures were made from a large number of corms in various stages of disease. The results of this study indicated that two fungi were causing most of the decay, one a *Fusarium*, the other a *Penicillium*. The latter is the subject of this paper.

The corms having the *Penicillium* type of rot had rather firm but not hard, reddish brown, sunken spots, sometimes slightly roughened by irregularly concentric wrinkles, more or less irregular in size and shape, margins indefinitely blending with the normal epidermis. Some lesions had no visible surface growth; others had a scanty growth of white mycelium and small sclerotia. Sections of such lesions showed the brown rot extending deeply and without definite margins into the flesh of the corms. The texture of the rotted tissues was only slightly porous, moderately firm and dry, but never dense and hard as in "hard rot." With a lens some white mycelium was seen in small cavities. In some of the corms sclerotia were found embedded in the tissues.

These sclerotia were smooth and cream colored, but in contrast to the brown tissues by which they were rather closely surrounded they appeared white. The sclerotia were found only in the older parts of the lesions. (Pl. 1, E.) Other corms with the same type of lesions had no sclerotia.

For cultures, bits of tissue were taken from the inner margin of lesions on corms with and without sclerotia. All of the cultures produced the same type of growth in which numerous sclerotia developed. The cultures seemed contaminated with a *Penicillium*, and numerous unsuccessful attempts were made to separate the sclerotia-forming fungus from the *Penicillium*. Sclerotia were isolated, sterilized by dipping in alcohol to remove air, then in mercuric chloride, 1:1,000, for periods up to 20 minutes. After being rinsed in sterile water, some of the sclerotia were placed at once on culture media; others were kept under dry conditions for several weeks, then put on culture media. Isolated single hyphae tips were used and also single conidia of the *Penicillium*. All of these sources of inoculum regularly and persistently produced both *Penicillium* conidia and sclerotia.

<sup>1</sup> Received for publication Nov. 14, 1927; issued April, 1928.

In June, 1926, the same type of lesion was found on gladiolus corms grown in New Mexico, and cultures from these produced *Penicillium* and sclerotia with the same characteristics as those from the Netherlands corms. Very shortly afterwards the same type of growth was also isolated from diseased *Tigridia* bulbs grown in New York. The gladiolus corms from New Mexico and the *Tigridia* bulbs were in more advanced stages of decay than the Netherlands corms, and both lots of material had numerous surface as well as interior sclerotia.

Another series of experiments for separation of the *Penicillium* and the sclerotia resulted in failure; so finally it seemed quite certain that the two forms belonged to the same fungus.

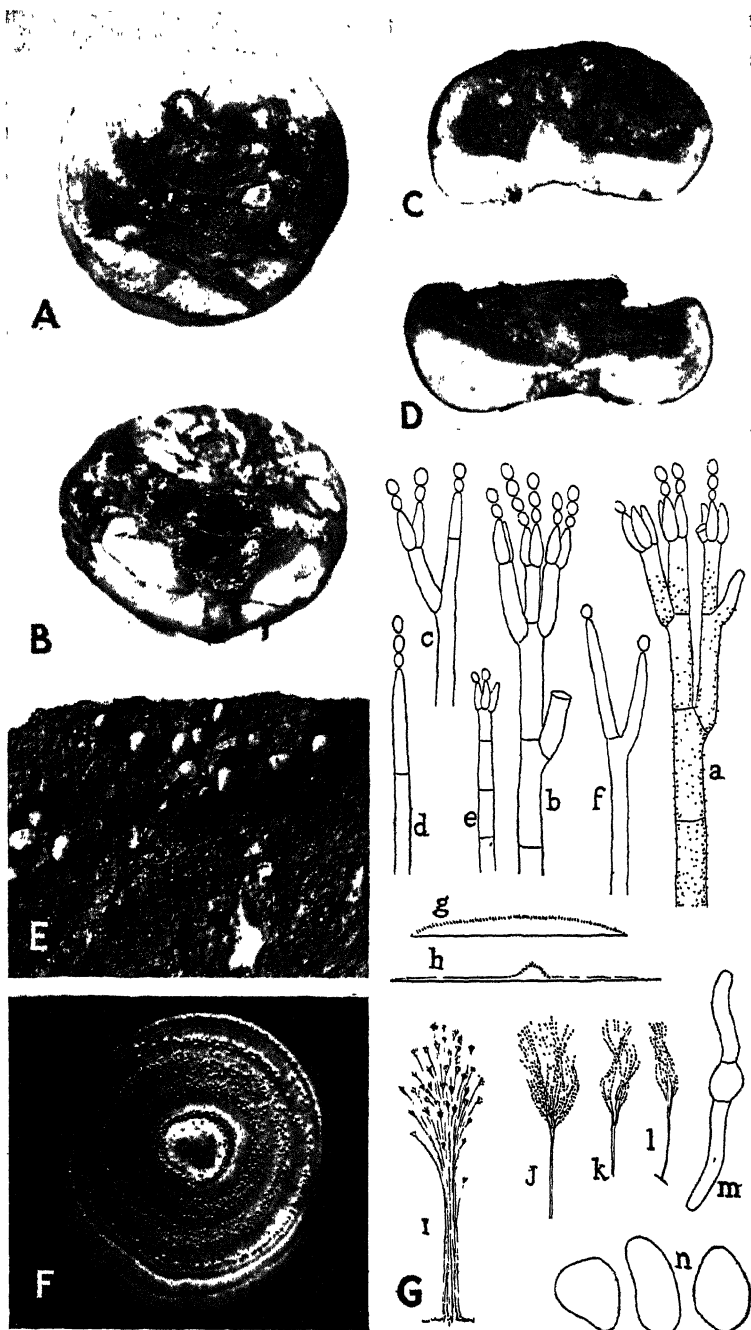
#### INOCULATION EXPERIMENTS

A number of perfectly clean gladiolus corms of the varieties Peace and America were husked, dipped in alcohol, covered with mercuric chloride for 10 minutes, rinsed well in sterile water, and then placed separately in sterile, covered dishes. The corms were inoculated with pure cultures of the fungus, using young mycelium not yet producing sclerotia for some, and for others older cultures producing sclerotia. One-half of the corms had the inoculum placed on the intact epidermis. The other half had small wounds made with a sterile scalpel and the inoculum placed over the injured tissue. After five weeks at 22°-24° C. the fungus had failed to penetrate the epidermis of the unwounded corms. The wounded corms, on the contrary, soon showed a slight growth about the wounds and a discoloration which spread out fairly rapidly over the surface. This discoloration was a dull brown in color (Dresden brown and umber to sepia in Ridgway's colors) (4).<sup>2</sup> There was never a luxuriant growth on the surface of the corms, and only a few tufts of the dull gray-green *Penicillium* developed about the wounded areas. (Pl. 1, A, B.) Sections of these corms showed considerable penetration of the decay (pl. 1, C, D) and numerous sclerotia scattered through the dark-brown tissue. The Netherlands, New Mexico, and *Tigridia* strains of the fungus gave identical results.

Gladioli growing in pots were copiously inoculated by placing young cultures and sclerotia about the stems and directly on the growing corms. No wounds were made. These plants when mature, two months later, were entirely normal. A few inconspicuous-brown spots were found on various parts of some of the plants, but the *Penicillium* was never recovered in any of the cultures made from these spots.

In another experiment on gladioli in pots, opposite sides of large growing corms were inoculated. A section of the husk was carefully lifted and the inoculum placed directly on the epidermis. One side of each corm was wounded by pricking with a sterile needle, the husks were then replaced, and the corms re-covered with soil. Four weeks later, half of the corms were dug and examined. All the wounded areas showed infection as dark-brown spots with water-soaked margins. This dark-colored rot had penetrated 4-8 mm. into the flesh of the corm. Six weeks after inoculation the remaining corms were dug, and only the wounded sides showed infection. From the dark brown interior tissues of these inoculated corms the fungus was isolated in pure culture.

<sup>2</sup>Reference is made by number (italic) to "Literature cited." n. 224.



A, B, C, D.—Gladiolus corms inoculated with *P. gladioli*, four weeks after inoculation.  $\times 1$

E.—Sclerotia in corm tissues.  $\times 8$

F.—Thaxter agar colony.  $\times 1$

G.—a, Conidiophore from Czapek agar culture grown at 15° C.,  $\times$  about 650; b, grown at 22° C.,  $\times$  about 650; c, d, e, f, conidiophores in cultures grown at room temperature,  $\times$  about 650; g, cross section of colony grown at 15° C.,  $\times \frac{1}{2}$ ; h, colony grown at 22° C.,  $\times \frac{1}{2}$ ; i, coremia,  $\times 10$ ; j, k, l, penicillia,  $\times 45$ ; m, germinating conidium,  $\times$  about 650; n, sclerotia,  $\times 25$ . From camera lucida drawings



Tigridia bulbs were not used in any of the inoculation experiments' but the fungus isolated from the diseased Tigridia bulbs is culturally like that from the Netherlands and the New Mexico corms and it produces the same sort of decay and sclerotia when gladiolus corms are inoculated with it.

From these experiments it appears probable that this fungus is not dangerous except to wounded corms or to corms kept under very unfavorable conditions.

#### CULTURAL CHARACTERS

At average room temperatures (22°-24° C.) the fungus presents an entirely different aspect from that shown when grown at 14°-15°. At the higher temperature the mycelial and conidial development is more or less suppressed and the abundant sclerotia are the conspicuous feature of the growth. At the lower temperature the luxuriant growth of white mycelium and green conidia delays and often hinders the production of sclerotia.

Czapek's solution agar and potato-dextrose agar are favorable media for this fungus. Single sclerotia placed on plates produce in three days at room temperature areas of growth 3-5 mm. in diameter. The growth is dense and pure white except at the water-soaked margins. Sclerotia begin to develop about the sixth day in a zone surrounding the center of growth and continue to develop in successive zones as long as the colony grows. (Pl. 1, F.) As the sclerotia develop, the mycelium becomes thin and inconspicuous. In the areas where sclerotia are growing, the hyphae bear numerous drops of clear, pale orange-yellow liquid. Immediately following the production of the first sclerotia or at the same time there develops in the center of the colony a small tuft of the dull gray-green (mineral gray to tea green) (5) *Penicillium*. Colonies 40-60 mm. in diameter have *Penicillium* tufts 2-4 mm. in diameter. The reverse side of the growth when 2 to 3 weeks old is reddish brown (Sudan brown) (5) in the Thaxter agar plates and light pinkish cinnamon in the Czapek agar plates. In crowded plates where the colony growth is limited to 10 mm. or less the central tuft of *Penicillium* does not develop sufficiently to be visible, but conidia and conidiophores can be found by microscopic examination.

In tube cultures the growth is more luxuriant than on plates, and by the tenth day the numerous sclerotia form a continuous layer over the surface of the slant, the white mycelium showing scantily at margins and between sclerotia. *Penicillium* tufts may or may not be visible (conidiophores and conidia, sometimes small and imperfectly formed, can be found in a microscopic examination), and the drops of clear liquid are abundant.

Finally the white mycelium becomes scanty and inconspicuous, leaving the coarse, granular layer of closely packed sclerotia, with perhaps a small central area of dull green.

The individual sclerotia are palest yellow or a cream color, but in mass as seen in cultures they are pale pinkish tan. Light drab and drab are the closest matches found in Ridgway (5), but these are scarcely pink enough.

When grown at 14°-15° C. there is a luxuriant mycelial growth, and the whole surface is soon entirely covered with the bluish gray-

green conidia. The conidiophores bear large, definite heads, and coremia 1-4 mm. tall develop. Sclerotia are slow (three to five weeks) in forming, and in shallow layers of agar they often fail to develop. The conidial areas gradually lose their blue-green color, becoming olive gray and later dark brown, sepia to mummy brown (5).

On oatmeal, potato, malt, wort, oxalic acid, and whey agars, the growth is similar to that on potato-dextrose and Czapek agar. Shrinkage of the media often causes the growth to fall into irregular, wide folds. The medium is not discolored, except for a slight browning of beef agar and gelatin. The reverse colors of the growth vary from cinnamon buff to Sudan brown. The cultures have no odor.

On beef agar the growth is scanty, at first white but finally a cream color. The reverse color is yellow to reddish brown. There is a scanty development of conidia and few to no sclerotia.

Nutrient beef gelatin at 22° C. produces a moderate amount of white surface growth, with a bright reddish brown substratum. Liquefaction is very slow. The addition of 1 per cent saccharose did not improve the growth or hasten liquefaction.

Plain gelatin (15 per cent) in tap water produced very scanty growth and no liquefaction in two months.

Lactose agar (synthetic base and colored with brom cresol purple) produced only moderate mycelial growth but numerous sclerotia. The conidial fructification was a brighter green (celandine green to artemisia green) (5) than on most culture media. The reaction was acid.

Litmus milk cultures first redden the cream rim. Whey forms on the surface, but there is no evidence of curd. The clearing gradually extends downward and is complete in two weeks at 22°-23° C., the whole of the medium becoming translucent and a dull wine color.

Sterilized gladiolus corms were cut in half and inoculated with the fungus. Half were kept at 14° C. and half at 25°-26°. At the lower temperature the corms in three to four weeks were covered with a blue-green layer of conidia, which persisted for weeks, while at the higher temperature only scanty white mycelium, short and dense, developed. The gladiolus tissues were completely destroyed in two months, and sclerotia were produced in large numbers at both temperatures.

Ripe oranges were pricked with a sterile needle and inoculated with the fungus. A dark brown, only moderately soft, rot developed. After one month at room temperature the rot had spread from the small inoculated area throughout the whole orange. The rind was dark brown, but there was no surface growth of fungus except at the inoculated point, where there were a few gray-green tufts and some small, white, immature sclerotia. At 14° C. the rind was less dark in color and there was a luxuriant surface growth of bluish gray-green conidia on isolated conidiophores and coremia and also a number of immature white sclerotia. Examination showed that at both temperatures the mycelium had penetrated to all parts of the interior, but without causing any particular change in the appearance or odor of the oranges. Sclerotia were very abundant in the tissues of the rind. The fungus did not penetrate uninjured orange epidermis.

On most of these different media, cultures were grown at room temperature and also at 14°-15° C. At the lower temperatures the conidial growth is definite and usually abundant, while at the higher



temperatures conidial production is scanty and in some cases almost absent. Beef medium at room temperature has very few and very poorly developed conidiophores. (Pl. 1, G, *d, e, f.*) However, at room temperature the chains of spores are longer and resist breaking up better than those formed at 14°–15°. In favorable media at the lower temperature the conidiophores are taller and coarser and more branched than at room temperature, and the walls are finely roughened. (Pl. 1, G, *a.*) At the higher temperatures the same media produce smooth-walled conidiophores. (Pl. 1, G, *b, c.*)

#### TEMPERATURE RELATIONS

Parallel plate cultures of potato-dextrose agar were made and kept at 14°, 22°–23°, and 30° C. At the lower temperature growth is slower than at 22°–23°, but, continuing for a longer period, eventually covers more surface, producing dense mycelium and abundant conidia not only in the center of the medium but over all but the extreme margin. Sclerotia did not develop until after three weeks.

In three weeks at 22°–23° C. the medium was drying out and the thin creamy-white growth was thickly sprinkled with numerous sclerotia, the central area of gray-green being 3–5 mm. in diameter. At 30° a few hyphae developed on the bits of inoculum, but growth never spread out until after three weeks, when the temperature dropped to 27° and a scanty growth pushed out into the surrounding medium. Tube cultures grown at the same temperatures gave comparable results.

Sterilized gladiolus corm tissues were inoculated with bits of mycelium and grown at 5°, 10°, 15°, 20°, 25°, and 27° C. Five days after the inoculations there was at 5° no visible growth; at 10° very scanty white growth; at 15° a moderate amount of white mycelium and a small green area; at 20° better growth and more green color than at 15°; at 25° less growth and less green than at 15° and 20°, sclerotia present; at 27° no visible mycelium, no green color, sclerotia present.

In 15 days at 5° C. there were scanty white mycelium, no conidia, no sclerotia, and no discoloration of the medium. At 10° all exposed surfaces of the medium were covered with growth, bluish gray-green with pure white margins; no sclerotia present; medium not discolored; conidiophores in coremia 1–2 mm. tall, columns not very definite, white or colorless; only a trace of roughness on walls of the conidiophores. At 15° conidial development was more luxuriant than at 10°; numerous drops of pale yellow to reddish orange liquid; coremia 2–4 mm. tall, lower half of the columns pinkish to orange red; sclerotia numerous; conidiophores definitely rough walled; medium discolored and porous. At 20° there was less conidial development than at 10°, a few poorly developed coremia present; mycelium not visible; all exposed surfaces covered thickly with sclerotia; walls of conidiophores only slightly or not at all rough; medium discolored and some sclerotia forming in the parts just below the surface growth. At 25° surfaces were covered with sclerotia, scanty green color, but well-developed conidiophores were found. Evidently the gladiolus tissue is a more suitable food than any of the artificial media, which produced very poor conidiophores at 25°. At 27° a short dense layer of dull white mycelium and small sclerotia were observed, covering about one-

fourth of the exposed medium; there was no green color; no conidio-phores were found in microscopic examination; the medium was discolored and porous.

#### VITALITY

Potato-dextrose agar tube cultures, kept at room temperature and becoming rather dry, were alive at the end of 10 months, but were evidently reduced in vitality, for quite a number of the 10-month-old cultures failed to develop growth when transferred to fresh media. Cultures 6 to 9 months old readily renewed growth when transferred to fresh media.

Sclerotia, sterilized in 50 per cent alcohol for 2 minutes, then in  $\text{HgCl}_2$  1-1,000 for 15 minutes, were slow in renewing growth. Several started growth after the thirteenth day, and one remained dormant 40 days before showing signs of life. Sclerotia sterilized 20 minutes in  $\text{HgCl}_2$  1-1,000 showed the first growth 9 days later; by the twelfth day all were growing.

Sclerotia taken from dry corms, sterilized in alcohol two minutes, in  $\text{HgCl}_2$  1-1,000 for three minutes, rinsed well, and placed on favorable media, promptly produced growth. Some of these sterilized sclerotia were kept dry in a tube for 12 weeks, then put on media, but none of these produced growth.

Sclerotia from diseased corms that had been stored at room temperature for 11 to 12 months did not grow when placed in favorable media.

Dry sclerotia treated with 0.25 per cent aqueous solutions of Uspulun and Semesan up to 45 minutes, then rinsed several times in water, produced growth. Similar treatment was given to mature but still moist sclerotia, with the same results. Dry sclerotia treated with mercuric chloride 0.1 per cent (1-1,000) in water for 15 minutes were mostly killed. After 30 minutes' treatment no growth resulted. But moist sclerotia were much less affected by the same treatment. Growth was delayed but not destroyed by even 45 minutes' treatment with the mercuric chloride. This difficulty in killing the fungus, even when it is possible to apply the fungicide directly to the sclerotia, indicates the probable lack of success of control measures based on the sterilization of infected corms.

#### SOME OTHER SCLEROTIAL DISEASES

Several other known diseases of gladioli are caused by sclerotia-forming fungi. The dry-rot disease described by Drayton (2) is apparently definitely pathogenic to growing plants. No spore stage has been found for this fungus. The sclerotia are black and much smaller than those of *Penicillium gladioli* L. McC. and Thom.

*Aspergillus ochraceus* Wilhelm (7, 9) has been isolated by the senior writer from diseased gladiolus corms. The sclerotia formed by this fungus are larger and darker than those of *Penicillium gladioli*, and they are always accompanied by the typical spore stage of the *Aspergillus*.

From diseased Ixia corms the senior writer isolated a fungus which causes rapid and extensive rot of gladiolus corms under storage conditions. Numerous large dark-brown sclerotia develop, but no spore stage has been found. The identity of this fungus has not been determined. It may be identical with *Sclerotium tuliparum* Klebahn (3).

Another fungous disease of gladioli producing black sclerotia has been described by Voglino (8) and named *Sclerotinia bulborum*.

Schwartz (6) has reported a sclerotium-producing *Penicillium* which he and Wehmer identify as *P. italicum*. There seemed a possibility that the *Penicillium* of the gladiolus corms might be the species described by Schwartz. However, a study of a culture of this particular *P. italicum*, obtained from Schwartz, shows that it is culturally and morphologically unlike *P. gladioli*.

### TECHNICAL DESCRIPTION<sup>3</sup>

#### PENICILLIUM GLADIOLI

Colonies presenting two aspects: When grown at temperatures above 20° C., predominantly consisting of sclerotia with few and inconspicuous conidiophores; when grown at 15° C. or lower, showing abundant green conidial areas with delayed or partially suppressed sclerotium formation. Upon Czapek's solution agar at 20° to 24° C. producing a thin aerial felt of mycelium with sclerotia beginning about the sixth day and developing in successive concentric zones, and giving the characteristic appearance of the species; sclerotia 140 $\mu$  to 540 $\mu$  in diameter, at first cream to light pinkish tan, in age very pale brown or tan, smooth, and composed of thick-walled cells 8 $\mu$  to 12 $\mu$  in diameter, retaining their vitality several months; reverse light pinkish cinnamon; drops of orange-yellow fluid more or less conspicuous; odor none, conidiophores few, scattered and inconspicuous among the sclerotia, often very long (up to 2 mm.) and about 2 $\mu$  to 3.6 $\mu$  in diameter, later developing in more or less conspicuous tufts, fascicles, or complex branching coremia in the center of the colony and definitely green (bluish gray-green); penicillus consisting of the main axis of the conidiophore with or without one or two branches, bearing few metulae 10 $\mu$  to 12 $\mu$  long and verticils of few sterigmata 12 $\mu$  to 14 $\mu$  by 1.5 $\mu$  to 2 $\mu$  with tapering rather than acute points, and conidia elliptical-fusiform, smooth, hyaline, 2.8 $\mu$  to 3.6 $\mu$  by 2.5 $\mu$  to 3 $\mu$ , adhering in long chains in fluid mounts, more or less parallel then tangled as seen in the penicillus; swelling to 6 $\mu$  or 7 $\mu$  in diameter and germinating by one or two tubes.

Colonies grown at 14° to 15° C., producing abundant mycelium and conidial areas gray-green (light dull glaucous blue, glaucous blue, and greenish glaucous blue of Ridgway (5)), to the very margin of the colony; sclerotium formation delayed and reduced, not dominating the growth; conidiophores partly simple, partly aggregated into coremia, tending to be longer and coarser, and when in coremia commonly 3 $\mu$  to 4.5 $\mu$  or even 6 $\mu$  in diameter with walls pitted or roughened; penicillus coarser, more branched, and with larger verticils of sterigmata and the elements varying in diameter but following the conidiophore in being coarser than in the colonies grown at higher temperature; conidia not different from the conidia produced at higher temperature.

The conidial form described and figured here complies closely with the description of *Penicillium divergens* of Bainier and Sartory (1) and would have been identified as that species except for the entire lack of sclerotia in that species.

Found as a cause of decay in gladiolus corms. Specimens of natural and artificial infections on gladiolus corms and dry cultures have been deposited in the herbarium of the Office of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture.

#### SUMMARY

A disease of gladiolus corms and Tigridia bulbs caused by *Penicillium gladioli* is described. As known at present it is chiefly a storage disease. Experiments have proved that the fungus under ordinary conditions does not penetrate the normal uninjured epidermis of gladiolus corms, but through even slight wounds it rapidly invades the inner tissues of both mature and growing corms, causing a dark brown, moderately dense rot. Numerous small white to cream-colored sclerotia are usually formed in the rotted tissues.

<sup>3</sup> This description embodies and supplements that given by McCulloch and Thom in an earlier publication (4).

The fungus invades the tissues with about equal rapidity at temperatures ranging from 15° to 23° C. At the lower temperatures there is abundant blue-green conidial growth and delayed formation of sclerotia, while at the higher temperatures there is scanty or no visible conidial development, but a rapid and abundant production of sclerotia.

Experiments with mercuric chloride and commercial fungicides show that the sclerotia are rather resistant.

#### LITERATURE CITED

- (1) BAINIER, G., and SARTORY, A.  
1912. ÉTUDE DE DEUX *PENICILLIUM* NOUVEAUX PRODUCTEURS DE PIGMENT.  
Bul. Soc. Mycol. France 28: 270-279, illus.
- (2) DRAYTON, F. L.  
1926. THE DRY ROT DISEASE OF GLADIOLI. *Sci. Agr.* 6: 199-209, illus.
- (3) KLEBAHN, H.  
1905. ÜBER DIE BOTRYTISKRAKHEIT UND DIE SCLEROTIEN KRAKHEIT DER TULPEN. *Jahrb. Hamburg. Wiss. Anst. (Beiheft 3)* 22: 1-22, illus.
- (4) McCULLOCH, L., and THOM, C.  
1928. A CORM ROT OF GLADIOLUS CAUSED BY A *PENICILLIUM*. *Science* 67: 216-217.
- (5) RIDGWAY, R.  
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.
- (6) SCHWARTZ, W.  
1927. ÜBER EIN *PENICILLIUM* MIT FERTILEN SKLEROTIEN. *Ber. Deut. Bot. Gesell.* 44: 648-652.
- (7) THOM, C., and CHURCH, M. B.  
1926. THE *ASPERGILLI*. 272 p., illus. Baltimore, Md.
- (8) VOGLINO, P.  
1925. I FUNGHI PARASSITI PIÙ DANNOSI PIANTE COLTIVATI OSSERVATI NELLA PROVINCIA DI TORINO E REGIONI VICINE NEL 1924. *Ann. R. Accad. Agr. Torino* (1924) 67: 93-99.
- (9) WILHELM, K. A.  
1877. BEITRÄGE ZUR KENNTNISS DER PILZGATTUNG *ASPERGILLUS*. 70 p. Berlin. (Inaug. Diss.)

# LILAC BLIGHT IN THE UNITED STATES<sup>1</sup>

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## INTRODUCTION

In the summer of 1925 some blighted lilac twigs were sent to the pathological laboratory of the Bureau of Plant Industry by H. W. Anderson, of Urbana, Ill., with the inquiry whether this was the bacterial disease described by Van Hall from the Netherlands and hitherto unreported in the United States. The twigs were blackened wholly or on one side only; cross sections showed the discoloration extending sometimes only through the cortex but often involving the entire thickness of the stem. The discolored tissues of the cortex swarmed with bacteria. Poured plates yielded practically only one type of colony. This was round, white, and caused green fluorescence in the agar, but its most striking characteristic was a wrinkling of the surface, which began on the second day and was persistent. As no mention was made of this very noticeable character by Van Hall (2)<sup>2</sup> or by E. F. Smith in his unpublished notes it seemed unlikely that this was the organism with which they had worked. Good infections, however, were obtained with subcultures from these wrinkled colonies. Since no culture of *Bacterium syringae* (Van Hall) EFS. (*Pseudomonas syringae* Van Hall) was available for comparison, cultural work was carried on and the cultures compared with the brief published and unpublished descriptions of *Bact. syringae*. The results were inconclusive, however, although there was no substantial disagreement aside from the colony character mentioned above.

In the spring of 1926, Westerdijk, of the Netherlands, sent the writer some blighted lilac twigs from which the causal bacterium was readily and repeatedly isolated. This organism was proved infectious by inoculations on lilac twigs. None of the colonies in any of the isolations and reisolations from this material showed any trace of wrinkling.

Cultural comparisons of the Netherlands and Illinois isolations were then made, but no real differences aside from that mentioned were found. The effects produced by inoculation with the two strains likewise showed no distinguishable differences. (Fig. 1, A and B.) It seems evident, therefore, that the two are strains of the same organism (*Bacterium syringae*) and that the Illinois disease is identical with the Netherlands disease.

While the work here reported was in progress a note appeared by C. O. Smith (7) in which he states that *Bacterium citriputeale*, the cause of citrus blast and black pit, is also probably responsible for a new lilac blight in California. To quote: "This investigation is

<sup>1</sup> Received for publication Oct. 3, 1927; issued April, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 235.

well in progress, and the results thus far indicate that the citrus blast, the avocado blemish, and the California lilac disease are closely related and probably are caused by the same organism." Com-

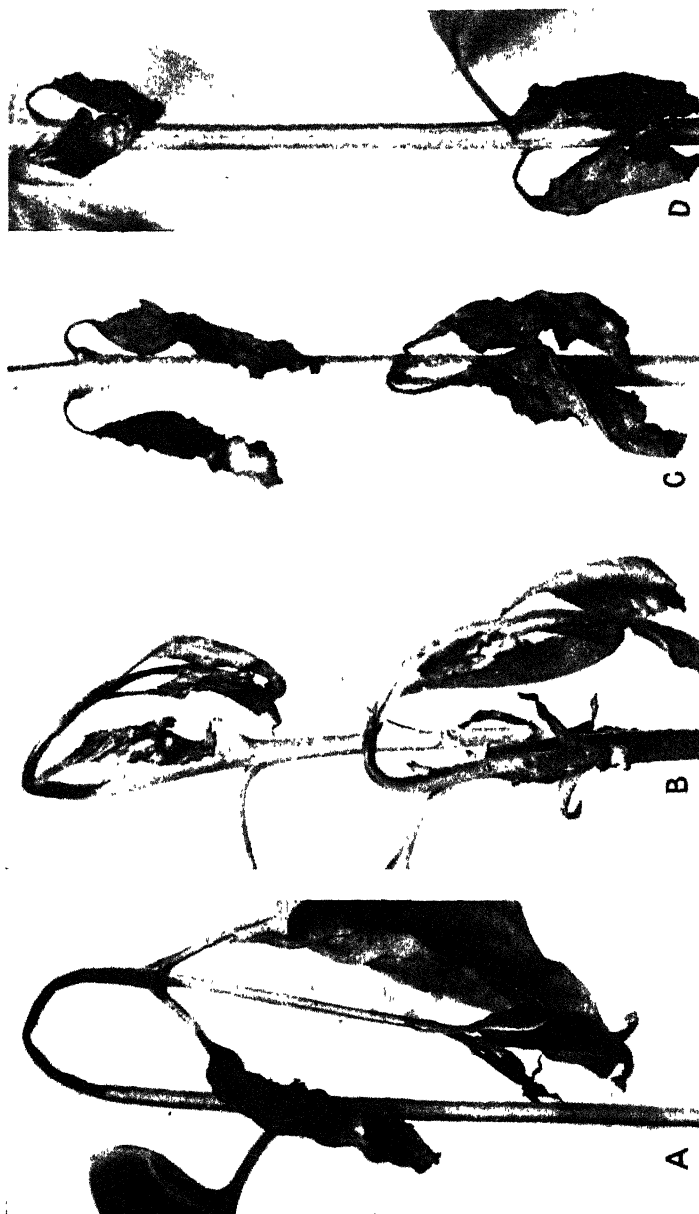


FIG. 1.—A, Lilac twig 12 days after prick inoculation with *Bacterium syringae* from the Netherlands; B, lilac twig 10 days after prick inoculation with *Bact. syringae* from Illinois; C, lilac leaves inoculated in the petioles with *Bact. syringae* from Illinois; D, lilac leaves inoculated in the petioles with *Bact. citriputeale*.

parisons were therefore made between *Bact. syringae* isolated from Illinois and Netherlands lilacs with a freshly isolated culture of *Bact. citriputeale* received from Fawcett in California. Parallel cultures proved the latter to be identical with *Bact. syringae* as sent

in colony characters on agar, and the differences in these were no more striking than the differences between the Netherlands and the Illinois strains. Inoculations on lilac with *Bact. citriputeale* gave good typical infections. (Fig. 1, D.) Good black-pit lesions were also produced on lemons by needle pricks with both the Netherlands and the Illinois strain; but these strains were somewhat less virulent on lemons than *Bact. citriputeale*.

From these comparisons it seems probable that the lilac blight of California is identical with that in Illinois and the Netherlands.

#### HISTORY OF THE DISEASE

Lilac blight has heretofore been reported only from the Netherlands, Germany, and England.

It was first observed by Sorauer (9) in Germany in 1891. In 1899 Ritzema Bos (5) found it in the Netherlands. Both considered it to be bacterial. Beyerinck subsequently obtained infections on lilacs with an organism which he isolated and studied. He did not publish, but turned over the organism and the results of his inoculations to Van Hall (2) who in 1902 published a detailed account of the appearance and progress of the disease. Van Hall made cultural studies of the bacterium, which he named *Pseudomonas syringae*, although he did not succeed in producing any infections with his organism.

Güssow (1) in 1908 published his observations on a lilac blight in England which he ascribed to *Pseudomonas syringae*.

In 1906 E. F. Smith while in the Netherlands studied this blight, made observations on its effect on the plant, and obtained good infections with the bacterium which he isolated, confirming Van Hall's work. He did not, however, publish the results of his studies.

#### APPEARANCE OF DISEASED PLANTS

As described by Van Hall (2), Sorauer (9), and E. F. Smith, the disease in Europe is identical in appearance and development with that in Illinois. Güssow's (1) description of the blight in England does not, however, conform to these descriptions. He does not mention stem or petiole infections, but confines his account to the appearance of the leaf blades, and even here the symptoms are unlike those of the Netherlands disease. His reported infections on leaf blades from needle pricks did not appear until two months after inoculation, while *Bacterium syringae* gives evidence of infection by the third day. It seems quite improbable, therefore, that he was dealing with the lilac blight of the Netherlands.

On the specimens received from the Netherlands in 1926 there were very distinct dark water-soaked angular spots on some leaves, usually on those with infected petioles. These spots swarmed with bacteria and yielded pure cultures of *Bacterium syringae* on plating. There were also lesions on the peduncles and pedicels of the flower clusters, and many flower buds were completely blackened.

In 1925, when Anderson first observed the disease in a large planting of lilacs in an arboretum in Illinois, their appearance suggested pear blight. Entire young shoots were blackened, some were bent, others erect. In some cases only one side of the shoot was involved,

ference of the stem in width extending the entire length of the season's growth. No definite leaf spots were observed, but leaves on the infected parts invariably were partially or entirely blackened.

In 1926 the disease did not appear at all in the planting of lilacs where it was found the previous year (see "Control," p. 232), but a less virulent outbreak occurred in the same arboretum on young nursery stock at a considerable distance from this planting. The season had been very dry, and when the plants were observed by the writer in July only small stem lesions from 5 to 15 mm. long could be found. Above and below these lesions, however, the leaves were rather thickly peppered with small irregular to round brown spots surrounded by a yellowish halo. (Fig. 2, A.) On the leaves nearest the stem lesion the leaf blades were ragged with large brown areas, due to coalescing spots and the torsion caused by irregular development of healthy and diseased areas. (Fig. 2, A.) The smaller spots were identical in appearance with those produced by inoculation in Washington. (Fig. 2, B and C.) (See "Inoculations.")

The disease attacks the young shoots as they develop in early spring, the first symptoms being brown spots on leaves and internodes. These become black and increase rapidly in size, especially in rainy weather. Further development depends largely on the age of the part attacked. On somewhat mature stems the spots usually enlarge longitudinally, passing over to the petioles in their path, causing the death of these leaves, but not of the remainder of the twig, which remains upright. When the spots occur on very immature internodes, on the other hand, the infection spreads around the twig, girdling it completely for a distance of several centimeters. (Fig. 1, A and B.) The stem bends over at this lesion, and the part above it withers and dies.

Similar differences are observable in the results of infection on immature and mature leaves. The immature leaves blacken rapidly and completely, while on older ones the spots enlarge slowly and at the worst, by coalescing, blacken and kill large areas of the blade, causing distortion.

#### TISSUES INVOLVED

The disease is primarily one of the parenchyma, although the vascular region may become involved. In the stem the bacteria spread through the intercellular spaces of the cortex, causing blackening and collapse of the cells and frequently cavities of greater or less extent. If the infection reaches the vascular system it may run up through this channel without external lesions, evidencing itself first by a wilting of the upper leaves, later by sunken blackened areas on the stem along the line of internal infection. In such cases, cavities filled with bacteria are formed in the vascular region. In infected petioles the parenchyma is very susceptible. The whole thickness of the petiole blackens; the bacteria make their way into the vascular system and are to be found far up in the midrib and veins, while the leaf blade blackens.

#### INOCULATIONS

Prick inoculations were made in the tender upper part of young lilac suckers in August, 1925, using subcultures from colonies isolated from the Illinois lilac twigs. In three days the stem near the pricks



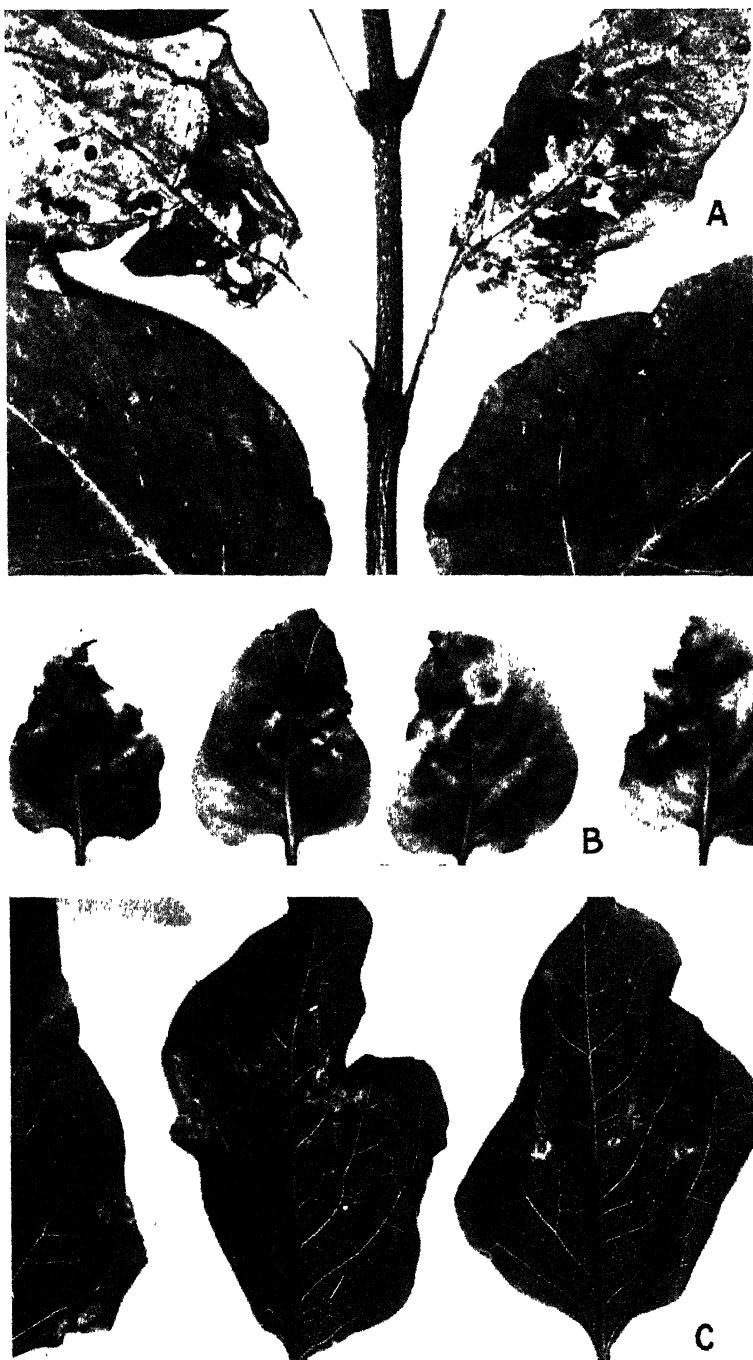


FIG. 2.—A, Natural infection on lilac leaves from Illinois, a small canker on the stem below the most seriously spotted leaves; B, lilac leaves 12 days after spraying with *Bacterium syringae* from Illinois; C, lilac leaves 14 days after spraying with *Bact. syringae* from Illinois

was blackened and the darkened area extended up into adjacent petioles. On the fourth day most of the inoculated shoots were completely dead and black above the point of inoculation. (Fig. 1, B.)

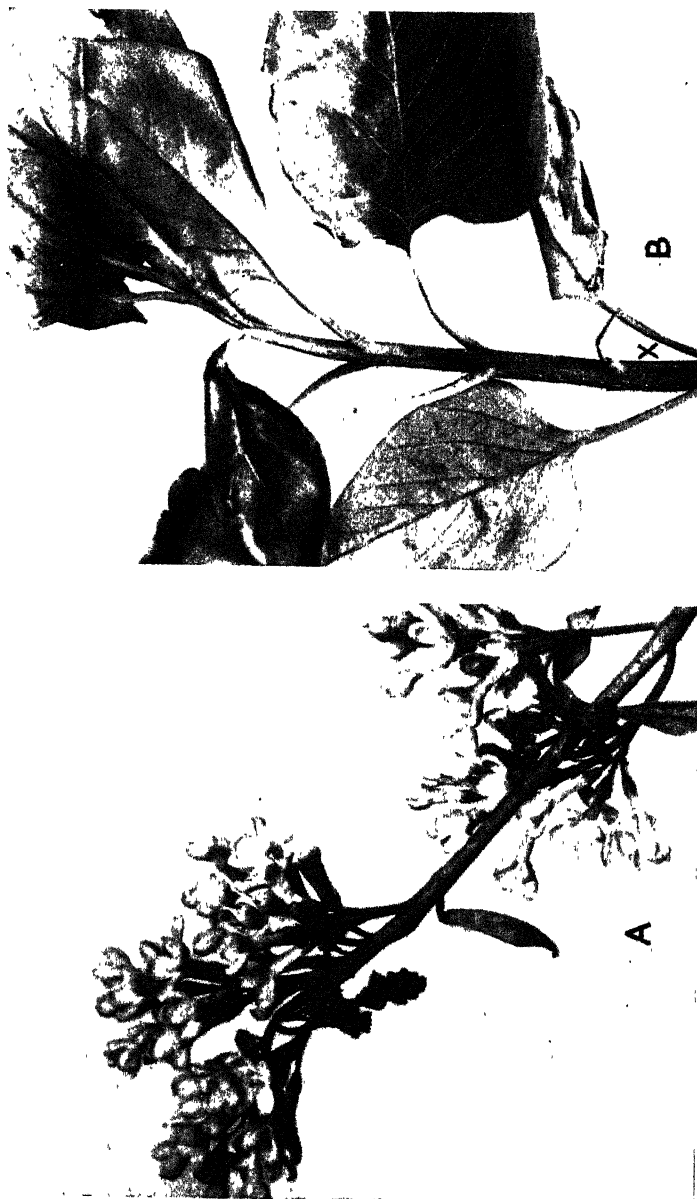


FIG. 3.—A. Infected flower clusters (the blackened drooping ones) inoculated by rubbing with *Bacterium syringae* from Illinois; B. lilac twig inoculated at X by needle pricks with Illinois strain of *Bact. syringae*; time, 16 days. The infection is advancing in the vascular region, showing in the surface lesions in two places and in the dying margins of the leaves

Isolations were made from one of these infections, and on August 25 subcultures were used to inoculate lilac shoots. Again infection was evident in two days, and after five days a dark streak extended downward through one or two internodes, invading the petioles in its

path but not causing a general blackening. Whether the whole twig blackened, or only a streak, seemed to depend largely on the age of the tissue inoculated. Pricks in the very tender tip always killed the twig; pricks made in slightly more mature wood gave only streaks. Sometimes infection spread in the cortex; in other cases it reached the vascular region quickly and spread rapidly up the stem through the vessels, as shown by the wilting of leaves in its path. Only later did a sunken black streak form externally along the line of internal infection. (Fig. 3, B.) Petioles were very susceptible, but infection did not often run back into the stem from them. When petioles became infected the whole leaf blade blackened, sometimes suddenly, at other times gradually.

The organism from the Netherlands, when used to inoculate lilacs in the pathological greenhouse, gave results identical with those obtained when the Illinois organism was used, except that the Netherlands organism seemed slightly more virulent. (Fig. 1, A.)

Stomatal infections on leaves were obtained by spraying and by rubbing with the Illinois organism and also by rubbing with the Netherlands isolations. Spots were round to irregular, brown, with a yellowish halo 1 or 2 mm. in width. The first evidence of infection was visible in five days, but the spots enlarged very slowly, causing slight distortion, and did not attain any considerable size under the very dry conditions prevailing during the lilac growing season. There was never any general spread of the disease from such infections. However, in the late fall of 1926, when rub inoculations were made on very tender leaves of young suckers during a period of rainy weather, infection spread so rapidly that individual stomatal infections were not observed. Leaves and petioles were rapidly and completely blackened. On most inoculated suckers there were also stem lesions 10 to 15 mm. long, black and sunken, and not continuous with diseased petioles, but apparently secondary stomatal infections. Isolations from these stem lesions gave wrinkled colonies from the stems inoculated with the Illinois organism and smooth colonies from those inoculated with the Netherlands organism.

#### SUSCEPTIBLE VARIETIES

The first observation of the disease in Illinois was on *Syringa vulgaris* only. In the nursery, however, it occurred on both purple and white sorts, *S. vulgaris* and the following varieties showing infection: Mont Blanc, Reine Elizabeth, Princess Alexandra, and Roi Albert.

Sorauer (9) reported that *Syringa chinensis* was less susceptible than other sorts, and Ritzema Bos (5) observed that *S. vulgaris* was more susceptible than *S. persica*.

#### ORIGIN OF THE DISEASE

An attempt to trace the infections in Illinois to importations failed, although there can be little doubt that the infection has at some time come to this country from Europe on nursery stock. Scions for the nursery where it was found came from several widely separated sources in the United States. No importations for this arboretum had been made from Europe except a few plants from France which had never been near either of the places in the arbore-

tum where infections were found, and none of the French importations showed the disease. Moreover, the disease is not known to exist in France.

### CONTROL

From the nature of the disease it is evident that sprays would be of little value and that destruction of diseased shoots is the only practical remedy. Careful cutting out was used in Illinois where the disease was severe in 1925, and there was no recurrence in 1926. The unusually dry season, however, may have been a factor in the completeness of its disappearance, since the disease elsewhere was very mild in its effects.

## THE ORGANISM

### CULTURAL CHARACTERS

Unless otherwise stated, all beef media used were made with beef infusion and had a  $P_H$  of 6.8 to 7.2.

**AGAR PLATES.**—On peptone beef-infusion agar, colonies of *Bacterium syringae* Netherlands are visible in 24 to 48 hours. These are round, white, smooth, convex, finely crosshatched, and opalescent by oblique transmitted light, transparent, attaining in six or seven days a width of 5 mm. and becoming flat, but otherwise retaining all of the characters mentioned above. There is no surface wrinkling at any stage. (Pl. 1, B, D, F, and G.) The agar becomes green.

Colonies of *Bacterium syringae* Illinois, on the same agar are visible in 24 to 48 hours and at this time are indistinguishable from colonies of the Netherlands strain. On the day after they appear the center has become sunken and holds one to several clear droplets. (Pl. 1, C.) By the third day the center is deeply wrinkled, these wrinkles increasing with the growth of the colony, while the margin remains raised, smooth, and finely crosshatched. Colonies may attain a diameter of 5 mm. by the sixth and seventh day. (Pl. 1, E and H.) The agar becomes green on all beef-infusion media.

Very occasionally in direct isolations of the Illinois organism, but frequently in plates from cultures, a very few smooth colonies appear; that is, some colonies retain the smooth surface of the first day and never show any wrinkling. These when used for further plating usually give only smooth colonies, but occasionally a few wrinkled ones appear.

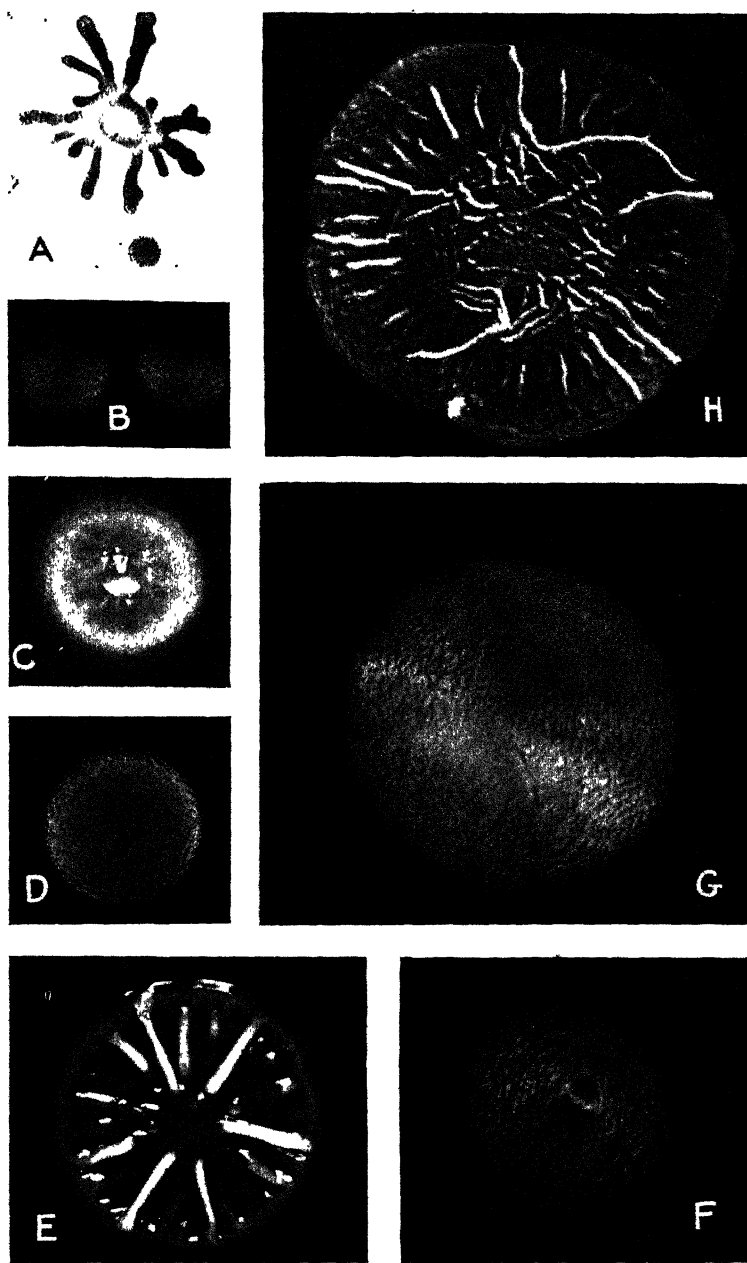
Rough and smooth strains of the same organism seem to be common to many organisms. The interconvertibility of the two types has recently been reported by Jordan (3) for certain animal pathogenes and by Sharp (6) for *Bacterium phaseoli sojense*. Rough colonies are not unusual in plates of smooth-colonied plant pathogenes. In this case the preponderance in the number of rough colonies is the unusual factor.

Occasionally spreading fimbriate colonies appeared on plates of the Illinois organism (pl. 2, A), never on those of the Netherlands organism. These colonies occasionally remained smooth but mostly became wrinkled. (Pl. 1, A.) Their occurrence seemed to depend on the surface tension of the agar, for they generally appeared on thicker portions of agar, due to an uneven bottom in the plate, and could be induced by using 20 c. c. instead of 10 c. c. of agar for a plate. This could not be done with the Netherlands strain.

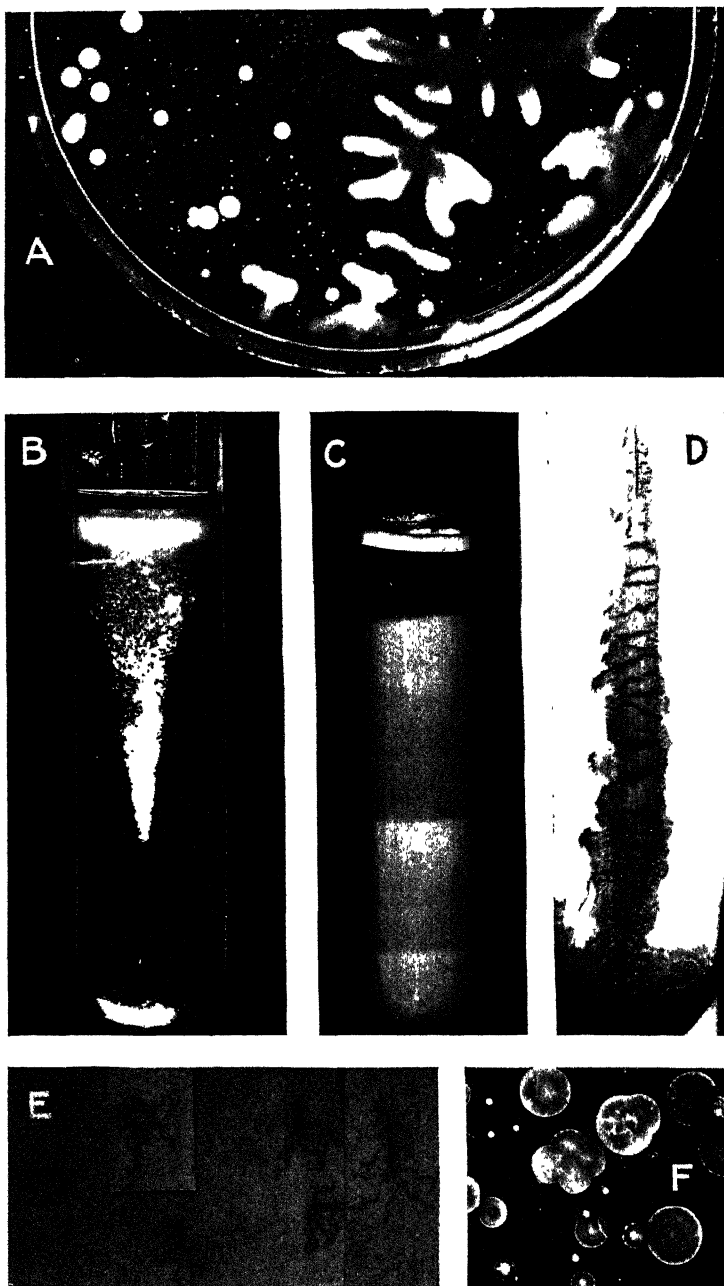
Occasionally colonies very irregular in outline, slightly roughened on the surface, and very coarsely crosshatched appeared with both the Netherlands and the Illinois isolations in platings from pure cultures. This form was very stable and has never been observed to revert to either of the other forms. It was very weakly pathogenic.

**BEEF-AGAR SLANTS.**—The same difference in surface in the two strains was apparent on beef-agar slants as on plates. (Pl. 2, D.) Growth is 3 to 7 mm. wide, white, transparent, opalescent, finely crosshatched by oblique light, with wavy or scalloped margins. The whole of the agar becomes green. Small crystals form in abundance just below the growth on the slant and below the V.

**BEEF-AGAR STABS.**—Growth characteristic of each strain occurs on the surface, finally covering it. In the stab, scanty growth is visible for less than one-third the length of the needle track. Small crystals form just below the surface of the agar.

Colonies of *Bacterium syringae* on beef-infusion agar

- A. Fimbriate colony, 4 days old, natural size  
 B. Illinois strain, 24 hours old. Colonies of the Holland strain are indistinguishable from these at this age  
 C. Illinois strain, 2 days old  
 D. Holland strain 2 days old  
 E. Illinois strain, 3 days old  
 F. Holland strain, 3 days old  
 G. Holland strain, 5 days old  
 H. Illinois strain, 5 days old  
 (B to H,  $\times 10$ )



A. Round and fimbriate colonies on beef-infusion agar plate, 2 days old  
 B. Gelatin stab, 48 hours old at 18° C.  
 C. Litmus milk culture, 4 days old  
 D. Beef-infusion agar slant, 3 days old  
 E. *Bacterium syringae* showing polar flagella  
 F. Colonies on beef-infusion gelatin plate, 2 days old

**BEEF BROTH.**—In peptone beef-infusion broth clouding is evident within 24 hours. Green fluorescence begins at the top on the second day, and a delicate filmy shining wrinkled pellicle forms which breaks into fragments readily on shaking. This pellicle is characteristic of wrinkled colonies only. Other types of colony have a dull white opaque pellicle which disappears as a fine cloud on shaking. All types produce the green fluorescence. Numerous small crystals lie in the white precipitate.

**BEEF-EXTRACT MEDIA.**—Growth is less vigorous on beef-extract agar than on beef-infusion agar, colonies attaining only approximately one-half the size under the same conditions for growth. No surface wrinkling occurs and no green fluorescence develops on plates, slants, or in broth made with beef extract, and only occasional small crystals are formed.

**POTATO CYLINDERS.**—On steamed potato cylinders growth is thin, grayish white, never becoming abundant. The potato is browned.

**COHN'S SOLUTION.**—There is very weak growth in Cohn's solution, evidenced by a faint but decided clouding and a very small quantity of white precipitate.

**USCHINSKY'S SOLUTION.**—Clouding is moderate to heavy in this medium, with a delicate rim and pellicle and a very pronounced green color. An abundant white precipitate is formed. No crystals were observed. The Netherlands strain gave heavier growth than the Illinois strain, as evidenced by both clouding and pellicle.

**FERMI'S SOLUTION.**—In Fermi's solution a heavy pellicle is formed, but the fluid is only moderately clouded and takes on a beautiful blue-green fluorescence.

**BLOOD SERUM.**—There is moderate growth on blood serum, but no liquefaction takes place.

#### PHYSIOLOGICAL CHARACTERS

**LIQUEFACTION OF GELATIN.**—In beef-peptone-gelatin stabs, made with both beef extract and beef infusion, liquefaction begins within 24 hours at 18° C. and is infundibuliform. Within a day or two it becomes stratiform at the top with a narrow funnel of liquefaction reaching one-half the depth of the gelatin or more and largely filled with growth. (Pl. 2, B.) When inoculations are made from freshly isolated cultures, liquefaction is rapid, often complete in 6 days, but when old isolations are used liquefaction progresses much more slowly and occasionally never is really complete, although the start is prompt and infundibuliform. The surface layer is green fluorescent. There is complete liquefaction and green fluorescence throughout in 6 to 16 days. With cultures which had been out of the plant for months, liquefaction began promptly at 18°, but progressed much more slowly and sometimes was not complete after six weeks.

**FERMENTATION OF SUGARS.**—Fermentation was tested on beef-extract agar and synthetic (ammonium phosphate) agar containing brom cresol purple with saccharose, dextrose, maltose, lactose, galactose, levulose, glycerin, and mannit. Acid was produced, beginning on the second day, from all except lactose and maltose, the agar becoming bright yellow throughout. Glycerin and mannit were somewhat less acid than the others. Cultures were held for two weeks. In the beef-extract agar, but not in the synthetic agar, alkali production began in five days and extended through two-thirds of the culture in saccharose, dextrose, galactose, and levulose. The two strains were alike with all the sugars tested.

In fermentation tubes containing 1 per cent peptone water with the same sugars there was no gas. Clouding appeared in the closed end with saccharose and dextrose only, heavier in saccharose in both strains.

**RELATION TO FREE OXYGEN.**—The organism is not strictly aerobic, since growth takes place in the closed end of fermentation tubes with saccharose and dextrose. In stab cultures, however, there is only scanty growth about one-third the depth of the needle track even when the sugars mentioned above are present.

**HYDROLYSIS OF STARCH.**—There is very slight diastatic action. Starch-agar plates seven days old, when flooded with iodine solution, show a purple area 3 to 8 mm. wide around the growth, while on potato cylinders growth is scanty.

**NITRATE REDUCTION.**—There is no nitrite reaction when 10-day-old nitrate beef-broth cultures are tested with the starch-potassium iodide-sulphuric acid method, but a very decided though very moderate nitrite reaction when the  $\alpha$ -naphthalymine-sulphanilic acid test was used. This would indicate very weak nitrate reduction.

**ACID IN MILK.**—No acid is formed in milk, but a slight alkalinity is evident in litmus milk.

**REDUCTION OF LITMUS.**—Litmus milk is blued slightly. A layer of clear whey 3 to 5 mm. forms at the top, but there is no further evidence of coagulation. Peptonization then begins and progresses downward in bands (pl. 2, C), followed by reduction of the litmus. Peptonization and reduction are complete within two weeks. Within the next two weeks the color returns to a dark grayish blue and remains so indefinitely.

**REDUCTION OF METHYLENE BLUE.**—Methylene blue in milk is reduced promptly and completely, the reduction beginning by the second day and becoming complete on the third or fourth day. By the fourth week the color returns a clear green (forest green according to Ridgway (4)).

**AMMONIA PRODUCTION.**—Ammonia production is strong in beef-peptone media.

**HYDROGEN SULPHIDE.**—Hydrogen sulphide is produced very scantily or not at all.

**PRODUCTION OF INDOL.**—No indol was produced in Dunham's solution within 20 days, tests being made at intervals of 5 days.

**TOLERATION OF ACID.**—Tests were made in beef-infusion broth and in beef-extract broth with a series of  $P_H$  values. Growth occurred in beef-infusion broth from  $P_H$  5.4 to  $P_H$  9.1, with the optimum at  $P_H$  6.8, and in extract broth from  $P_H$  5.3 to 9, with the optimum at  $P_H$  6.5 to 7.

**TOLERATION OF SODIUM CHLORIDE.**—There is clouding within 24 hours in beef broth containing 1, 2, and 3 per cent NaCl, in 2 days in 4 per cent, and after 5 days in 5 per cent. Very slight growth takes place in 6 per cent. In 3 to 6 per cent NaCl growth consists of distorted irregularly swollen filaments.

**RESISTANCE TO DESICCATION.**—The organism is very sensitive to drying. Drops of a 24-hour-old culture placed on sterile cover glasses in sterile Petri dishes and kept in the dark gave no clouding when dropped into beef broth after 2 days' drying.

**TEMPERATURE RELATIONS.**—Best growth occurs at 28° to 30° C. Beef-broth cultures kept at 35°, 36°, and 37° for 8 days remained clear. When removed to room temperature (28° C.) the 35° cultures clouded but the 36° and 37° cultures remained clear. Within 24 hours clouding was visible in beef-broth cultures at temperatures from 10° to 30° and after 5 days at 1° to 2.5°. The thermal death point is 51° for both strains.

#### BRIEF CHARACTERIZATION OF BACTERIUM SYRINGAE<sup>3</sup>

*Bacterium syringae* is a motile rod 1.2 to 1.8 by 0.6  $\mu$  with one to several polar flagella; single, in pairs or short chains. Long twisted filaments and chains occur in salt bouillon. No spores are formed. A small capsule may be demonstrated by Ribbert's dahlia stain. The organism is Gram-negative, but stains readily with the ordinary bacterial stains. An irregular or polar staining is obtained with carbol fuchsin. Agar colonies round or fimbriate, smooth or wrinkled, white; liquefies gelatin rapidly; produces green fluorescence in beef-infusion media; reduces litmus and methylene blue in milk; forms a soft rennet curd in milk and clears it in bands; produces ammonia but no indol or hydrogen sulphide; weak nitrate reduction; feeble diastatic action on starch; grows well in Uschinsky's solution and Fermi's solution, but very feebly in Cohn's solution; produces acid from saccharose, dextrose, galactose, levulose, glycerin, and mannit, but not from lactose or maltose within two weeks; no gas; tolerates 5 per cent NaCl in beef broth; optimum temperature 28° to 30° C., maximum 35°, minimum below 1°.

#### SUMMARY

A lilac blight occurring in Illinois has been proved to be identical with the lilac blight of Europe caused by *Bacterium syringae* (Van Hall) EFS.

The disease blackens and kills young stems and leaves in the spring. It is most destructive in rainy seasons. The appearance of affected plants suggests fire blight.

The blight is primarily a disease of the parenchyma, but it also invades the vascular tissue.

Careful pruning out of diseased twigs is the best method of control. Cultural characters of the organism are given.

<sup>3</sup> "Bacterium" is here used according to the system of nomenclature proposed by Erwin F. Smith (8, p. 171) and used by him for this organism in the same volume (8, p. 63).



*Bacterium citriputale* has been found indistinguishable from *Bact. syringae* in cultures, and cross inoculations with the two organisms have produced good infections.

## LITERATURE CITED

- (1) GÜSSOW, H. T.  
1908. NEW LILAC LEAF DISEASE IN ENGLAND. (PSEUDOMONAS SYRINGAE). Gard. Chron. (3) 44: 404-405, illus.
- (2) HALL, C. J. J. VAN  
1902. BIJDRAGEN TOT DE KENNIS DER BAKTERIEELE PLANTENZIEKTEN. 198 p. Amsterdam.
- (3) JORDAN, E. O.  
1926. THE INTERCONVERTIBILITY OF "ROUGH" AND "SMOOTH" BACTERIAL TYPES. Jour. Amer. Med. Assoc. 86: 177-178.
- (4) RIDGWAY, R.  
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.
- (5) RITZEMA BOS, J.  
1899. EINE BAKTERIENZIEKTE DER SYRINGEN. Tijdschr. Plantenziekten 5: 177-183.
- (6) SHARP, C. G.  
1927. CORRELATION OF VIRULENCE AND ACID AGGLUTINATION OF A SMOOTH AND A ROUGH STRAIN OF BACTERIUM PHASEOLI SOJENSE. (Abstract) Phytopathology 17: 49.
- (7) SMITH, C. O.  
1926. SIMILARITY OF BACTERIAL DISEASES OF AVOCADO, LILAC, AND CITRUS IN CALIFORNIA. Phytopathology 16: 235-236.
- (8) SMITH, E. F.  
1905. BACTERIA IN RELATION TO PLANT DISEASES. v. 1, illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 27.)
- (9) SORAUER, P.  
1891. NEUE KRANKHEITERSCHREINUNG BEI SYRINGA. Ztschr. Pflanzenkrank. 1: 186-188.



# FURTHER EXPERIMENTS WITH SEED TREATMENTS FOR SWEET-CORN DISEASES<sup>1</sup>

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## INTRODUCTION

This paper presents the results from additional experiments with seed treatments for the control of certain seed-borne diseases of sweet corn (*Zea mays saccharata*). These experiments were conducted in 1926 in the greenhouse and field at the Arlington Experiment Farm, Rosslyn, Va., and in the field near Bloomington, Ill.<sup>3</sup>

A previous paper<sup>4</sup> on this subject dealt for the most part with experiments conducted in 1925. For several reasons, it was thought that the 1925 results from seed treatments for sweet-corn diseases were not indicative of the full possibilities or even the average possibilities of such treatments for increasing yields from diseased seed. The spring of 1925 was exceptionally dry, and the plantings were made late when the soil had warmed to a considerable degree. In moderately dry warm soil both *Diplodia zeae* and *Gibberella saubinetii* produce minimum effects as seedling blights of corn.<sup>5 6</sup>

The 1926 results, presented in this paper, were obtained for the most part under conditions favorable to the development of *Diplodia* seedling blight. For this reason, the addition of these results to those already published should give a basis for a more accurate estimate of the value of seed treatments for the control of certain sweet-corn diseases.

## PRELIMINARY SEED-TREATMENT EXPERIMENTS IN GREENHOUSE

One of the early difficulties encountered in experimenting with seed treatments for corn was to find a satisfactory means of limiting the number of seed-treatment materials to be used in field experiments without omitting possibly the best ones.

Short-time preliminary experiments were conducted in the greenhouse for this purpose. Cylindrical cans 8 inches in diameter and 10 inches in height were filled with moist soil to within 4 inches of the top. A wire cage or basket of 1/2-inch mesh was set on the soil and 2 inches of wet sand placed in this. Twenty-five kernels of the corn under experimentation were laid on this sand and then covered with another inch of wet sand. (Fig. 1.) Four cans were used for each treatment and more than four for each control.

<sup>1</sup> Received for publication Nov. 29, 1927; issued April, 1928.

<sup>2</sup> The writers wish to express their indebtedness to A. G. Johnson, of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, for advice, helpful suggestions, and assistance in preparing the manuscript.

<sup>3</sup> The investigations conducted near Bloomington, Ill., were in cooperation with Funk Brothers Seed Co. <sup>4</sup> REDDY, C. S., HOLBERT, J. R., and ERWIN, A. T. SEED TREATMENTS FOR SWEET-CORN DISEASES. Jour. Agr. Research 33: 769-779, illus. 1926.

<sup>5</sup> HOLBERT, J. R., BURLISON, W. L., KOEHLER, B., WOODWORTH, C. M., and DUNGAN, G. H. CORN ROOT, STALK, AND EAR ROT DISEASES, AND THEIR CONTROL THRU SEED SELECTION AND BREEDING. Ill. Agr. Expt. Sta. Bul. 255, p. 239-478, illus. 1924.

<sup>6</sup> DICKSON, J. G. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF THE SEEDLING-BLIGHT OF WHEAT AND CORN CAUSED BY GIBBERELLA SAUBINETII. Jour. Agr. Research 23: 837-870, illus. 1923.

Usually it was not necessary to add water during the period of the experiment, which was about 16 days. When necessary, an equal quantity of water was added to each can. The seed used was Country Gentleman sweet corn, which, on the germinator, had shown 88 per cent viability and 50 per cent infection with *Diplodia zeae*. Wire cages of somewhat smaller mesh might have been better than those used. In similar experiments with dent corn, conducted by the junior writer, the procedure was varied from that described above by adding the sand in a dry condition, moistening with measured quantities of water, and then adding a light sprinkling of dry sand to serve as a mulch.

When *Diplodia*-infected seed is used, the most satisfactory results are obtained if greenhouse temperatures are between 22 and 25° C.

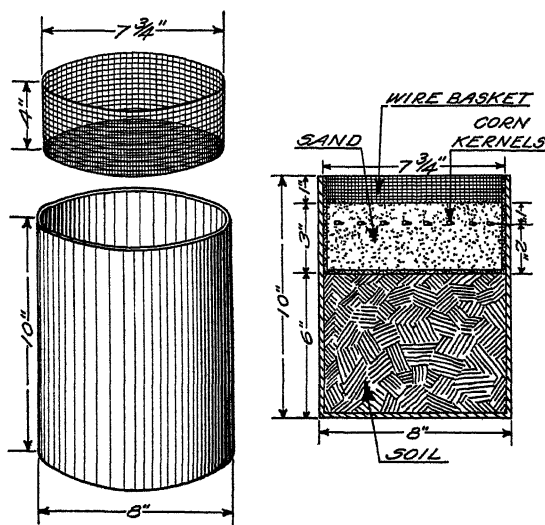


FIG. 1.—Soil can, wire basket, and diagram showing their use in preliminary seed-treatment experiments

and the moistures are kept at 60 to 65 per cent of the water-holding capacities of sand and soil. These conditions insure maximum germination of *Diplodia*-infected seed, good early growth, and the greatest development of *Diplodia* seedling blight.<sup>7</sup> Moderately high soil moisture is very important, but it should not exceed 67 to 70 per cent of the water-holding capacity.

When *Gibberella*-infected seed is used, the greenhouse temperature should be

maintained at 15 to 17° C., with the soil moisture about the same as or somewhat lower than for the *Diplodia*-infected seed.

The seed-treatment materials and methods used were:

- |   |   |
|---|---|
| 1. Uspulun, 0.5 per cent, one and one-half hour soak.   | 13. Du Pont 12 Bel B, dust.                                 |
| 2. Semesan, 0.5 per cent, one and one-half hour soak.   | 14. Du Pont 35, dust (included only for field experiments). |
| 3. Mercury A, 0.5 per cent, one and one-half hour soak. | 15. Du Pont 50, dust.                                       |
| 4. Bayer, dust.   | 16. Du Pont 51, dust.                                       |
| 5. Semesan, dust.                                       | 17. Du Pont 57, dust.                                       |
| 6. Semesan Jr., dust.                                   | 18. Du Pont 62, dust.                                       |
| 7. Semesan 13 UG, dust.                                 | 19. Du Pont 63, dust.                                       |
| 8. Semesan 13 UH, dust.                                 | 20. Du Pont Z4, dust.                                       |
| 9. Du Pont 12, dust.                                    | 21. Mercury C, dust.  |
| 10. Du Pont 12 Bel-10 (Semesan Bel), dust.              | 22. Corona 640, dust.                                       |
| 11. Du Pont 12 Bel-6, dust.                             | 23. Abavit B, dust.   |
| 12. Du Pont 12 Bel A, dust.                             | 24. S. F. A. 225, dust.                                     |
|   | 25. S. F. A. 225 V, dust.                                   |
|   | 26. S. F. A. AZ III, dust.                                  |
|   | 27. S. I. 220, dust.  |

<sup>7</sup> HOLBERT, J. R., KOEHLER, B., and DUNGAN, G. H. THE DIPLODIA DISEASE OF CORN. III. Agr. Expt. Sta. (Unpublished manuscript.)

Nos. 1 and 4 were obtained from the Bayer Co. (Inc.), New York, N. Y.; 2 and 5 to 20, inclusive, from E. I. du Pont de Nemours & Co. (Inc.), Wilmington, Del.; 3 and 21 from Roessler & Hasslacher Chemical Co., Perth Amboy, N. J.; 22 from Corona Chemical Division, Pittsburgh Plate Glass Co., Milwaukee, Wis.; 23 from Chemische Fabrik Ludwig Meyer, Mainz, Germany; 24 to 26, inclusive, from Saccharin-Fabrik, Actiengesellschaft, Magdeburg, Südost, Germany; and 27 from Actiengesellschaft für Anilin Fabrikation, Wolfen, Kreis Bitterfeld, Germany.

When the treatment controlled the disease without injury to the seed, the roots grew rapidly through the sand into the soil and an

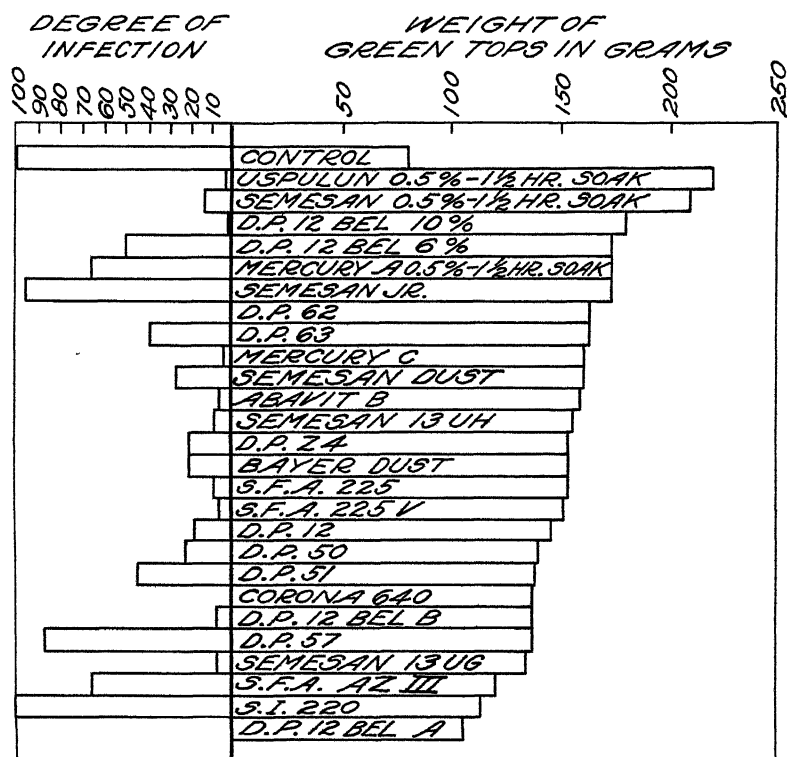


FIG. 2.—Results of preliminary seed-treatment experiments with *Diplodia*-infected Country Gentleman sweet-corn seed, showing the weights of green tops and the degrees of infection in proportion to those of the control (taken as 100)

early development of the aerial parts resulted. When no treatment was applied or when the treatment did not control the disease, the infected plants grew slowly. Often the roots did not reach the soil, and as a result the growth of the aerial portions was retarded. Sometimes the treatment controlled the disease but exerted a depressing effect on the growth of the plants. In this case also the top growth was retarded.

At the end of about 16 days the weights of green tops were obtained, the wire cages were removed, and the sand was washed out by a stream of water from a hose so that the parts of the plants below the surface of the sand were exposed for observation. Data then were

taken on the number and the severity of the infections. At this stage most of the lesions are on the mesocotyls.

The data from these preliminary experiments are presented graphically in Figure 2. In this graph the results, except those from the control series, are arranged in a graduated series according to the weight of green tops obtained. The degree of infection is relative to the infection found in the controls, which is arbitrarily taken as 100. The plants are shown in Figure 3.

In these preliminary tests the best treatment materials are indicated by high weights of tops and low degrees of infection. In general, the results of these preliminary experiments indicated that

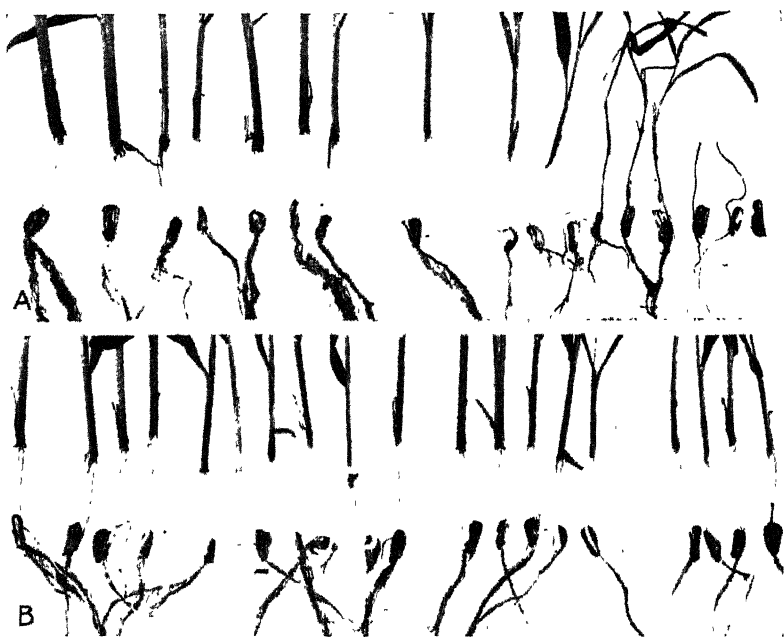


FIG. 3.—Country Gentleman sweet-corn plants, 16 days old, grown in the greenhouse from *Diplodia*-infected seed, using the method and apparatus illustrated in Figure 1, at Arlington Experiment Farm, Rosslyn, Va., January, 1927. A—Plants from untreated seed, showing all the plants from one of the control cans. Of the 25 kernels planted, 17 produced plants. Of these, 7 were uninfected, 1 slightly infected, 3 moderately infected, and 6 severely infected (dead). B—Plants from seed treated with organic-mercury dust showing all the plants from one can. Of the 25 kernels planted, 18 produced plants. Of these, 12 were uninfected, 5 slightly infected, 1 moderately infected, none severely infected (dead).

Uspulun and Semesan were practically equally efficient as liquid disinfectants. A number of dusts gave promising indications of control. However, some of the dusts seemed to show depressing effects on the growth of tops, or did not control infections, or showed both reactions.

The results from the last seed treatment (Du Pont 12 Bel A) represented in Figure 2 show that the disease was controlled, but that the weight of tops produced was lowest. This low weight of tops probably is the result of an injurious effect of the treatment. The treatment that resulted in the next lowest weight of tops permitted a high degree of infection. It is likely in this case that lack of disease control rather than an injurious effect on the plants caused the small development of tops.

TIME-OF-PLANTING AND SEED-TREATMENT EXPERIMENT  
IN FIELD

## MATERIALS AND METHODS

A combined time-of-planting and seed-treatment experiment was conducted in the field at the Arlington Experiment Farm, Rosslyn, Va., in which specially selected *Diplodia*-infected Country Gentleman seed grown at Bloomington, Ill., in 1925 was used. Plantings were made on April 6, 14, and 22, and May 7, 1926. Half of the seed was dusted with S. F. A. 225 at the rate of 2 ounces per bushel. The treatment was made by shaking the seed and the dust in a closed container for 10 minutes. Each planting consisted of 12 rows of 19 hills each, the hills being spaced 40 inches apart each way. Six rows were planted with treated seed, and untreated seed was used in alternate rows, making six replications in each of the four plantings. Three kernels were planted to the hill. Stand data were taken when the plants were about 6 inches high. Yield data were taken when the corn was prime for canning. Total yields were obtained by weighing the snapped ears without removing the husks. Yields of corn prime for canning were obtained after the husks were removed. These latter yields did not include small nubbins, rotten ears, and ears which had not reached or had passed the prime stage.

## SOIL MOISTURE AND TEMPERATURE RELATIONS

The daily maximum and minimum soil temperatures at a depth of 1 inch and the daily rainfall for the months of April and May are given in Table 1.

TABLE 1.—Daily maximum and minimum soil temperatures at a depth of 1 inch and daily rainfall for April and May, 1926, at Arlington Experiment Farm, Rosslyn, Va.

Date	Soil temperatures (° C.)		Rainfall (inches)	Date	Soil temperatures (° C.)		Rainfall (inches)
	Maximum	Minimum			Maximum	Minimum	
Apr. 1.....			0.07	May 1.....	30	11	
Apr. 2.....				May 2.....	30	13	
Apr. 3.....				May 3.....	27	7	
Apr. 4.....			.02	May 4.....	24	3	
Apr. 5.....				May 5.....	27	7	
Apr. 6.....	15	2		May 6.....	27	11	
Apr. 7.....	17	9	.16	May 7.....	34	12	
Apr. 8.....	18	7	.17	May 8.....	33	12	
Apr. 9.....	17	2	.27	May 9.....	30	13	
Apr. 10.....	20	7		May 10.....	23	9	
Apr. 11.....	12	1		May 11.....	26	9	
Apr. 12.....	13	2	.17	May 12.....	28	13	
Apr. 13.....	8	3		May 13.....	30	9	
Apr. 14.....	18	4	.02	May 14.....	30	11	0.13
Apr. 15.....	15	0		May 15.....	30	12	2.41
Apr. 16.....	20	5		May 16.....	15	7	
Apr. 17.....	19	5		May 17.....	25	11	
Apr. 18.....	9	0		May 18.....	27	14	
Apr. 19.....	11	-1	.05	May 19.....	26	12	.15
Apr. 20.....	14	2		May 20.....	21	9	
Apr. 21.....	24	6		May 21.....	22	9	
Apr. 22.....	28	10		May 22.....	26	10	
Apr. 23.....	24	10		May 23.....	17	6	.01
Apr. 24.....	28	9		May 24.....	22	16	
Apr. 25.....	23	6	.01	May 25.....	30		
Apr. 26.....	21	3		May 26.....		8	
Apr. 27.....	24	9		May 27.....	25	12	
Apr. 28.....	15	3		May 28.....	26	12	
Apr. 29.....	21	4	.03	May 29.....	24	7	
Apr. 30.....	25	11		May 30.....	26	8	
				May 31.....	26	14	

Table 1 shows that the soil was cold for a number of days following the first planting, April 6. As a result, the first and second plantings, made April 6 and 14, emerged at the same time. It is of interest to note later the effect of these severe conditions at planting time on the field stands and yields from untreated and treated seed. In general, the early growth of the first series (April 6) was in cold, moderately moist soil; that of the second (April 14) and third (April 22) series

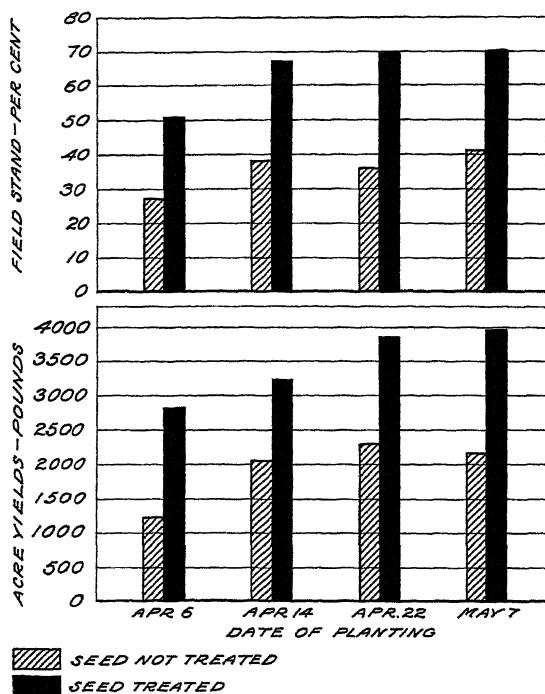


FIG. 4.—Field stands and acre yields of Country Gentleman sweet corn, prime for canning, with husks removed, grown from *Diplodia*-infected seed, untreated or treated with S. F. A. 225 dust and planted on four different dates at Arlington Experiment Farm, Rosslyn, Va., in 1926. (Data from Tables 2 and 3)

was in moderately cold, dry soil; and that of the fourth series (May 7) was in moderately warm, wet soil. If any of the series had been planted in warm, moderately dry soil, very little *Diplodia* seedling blight would have been expected to develop and, as a result, there would have been very little increase in the yields from treated seed. However, as these two conditions, namely, warm and moderately dry soil, did not obtain simultaneously during the critical periods of these experiments, it would be expected that *Diplodia* seedling blight would be active in each series and that significant increases in yield would be obtained from the treated seed, if the treatment controlled the disease without injuring the seed.

#### DATA ON STAND AND YIELD

The stand data from this seed-treatment experiment, which involves four series of plantings of untreated and treated *Diplodia*-infected Country Gentleman seed, are presented in Table 2 and Figure 4. The yield data are presented in Table 3 and shown graphically in Figure 4.



TABLE 2.—Mean field stands from *Diplodia*-infected Country Gentleman seed corn, untreated or treated with *S. F. A. 225* dust and planted at Arlington Experiment Farm, Rosslyn, Va., on four different dates in 1926

Date of planting	Soil conditions following planting	Field stand average of 6 replications (per cent)		
		Untreated seed	Treated seed	Increase due to seed treatment
Apr. 6.....	Cold and moderately moist.....	27.2	51.2	88.2
Apr. 14.....	Cold and moderately dry.....	38.0	67.9	78.7
Apr. 22.....	do.....	36.8	69.3	88.3
May 7.....	Warm and moderately wet.....	41.5	70.8	70.6

TABLE 3.—Acre and plot yields of snapped ears and of husked ears prime for canning, grown from *Diplodia*-infected Country Gentleman seed corn, untreated or treated with *S. F. A. 225* dust, as planted in six replications in 1926, at Arlington Experiment Farm, Rosslyn, Va.

Data compared	Untreated seed		Treated seed	
	Total ears, husks not removed	Ears prime for canning, husks removed	Total ears, husks not removed	Ears prime for canning, husks removed
Series 1, planted Apr. 6:				
Plot yields, 6 replications.....pounds..	15.8	5.1	29.5	13.0
	17.8	4.8	39.4	14.1
	15.8	6.8	37.8	12.6
	16.8	6.5	41.2	15.8
	15.3	6.5	41.0	13.0
	17.2	6.2	36.5	12.8
Total.....do.....	98.7	35.9	225.4	81.3
Mean acre yields.....do.....	3,463	1,280	7,909	2,853
Net increase.....do.....			4,446	1,593
Increase.....per cent.....			128.4	126.4
Odds.....			9,999:1	9,999:1
Series 2, planted Apr. 14:				
Plot yields, 6 replications.....pounds..	30.1	11.0	41.6	17.5
	25.0	9.0	48.3	19.6
	22.0	9.0	35.1	13.1
	24.2	9.0	36.0	16.0
	29.2	11.5	32.5	12.9
	24.0	8.8	33.5	13.2
Total.....do.....	154.5	58.3	227.0	92.3
Mean acre yields.....do.....	5,421	2,046	7,965	3,239
Net increase.....do.....			2,544	1,193
Increase.....per cent.....			46.9	58.3
Odds.....			328:1	295:1
Series 3, planted Apr. 22:				
Plot yields, 6 replications.....pounds..	24.5	12.2	32.5	18.2
	23.5	12.5	35.6	19.5
	22.5	13.1	31.3	16.8
	26.5	12.8	35.7	19.8
	13.7	6.0	26.7	14.9
	16.2	9.5	40.5	21.0
Total.....do.....	126.9	66.1	202.3	110.2
Mean acre yields.....do.....	4,453	2,319	7,099	3,867
Net increase.....do.....			2,646	1,548
Increase.....per cent.....			59.4	66.8
Odds.....			521:1	1,683:1
Series 4, planted May 7:				
Plot yields, 6 replications.....pounds..	19.6	8.6	39.5	19.8
	24.8	11.5	46.1	22.0
	26.8	11.5	38.0	18.1
	24.5	10.9	39.0	20.5
	19.0	9.0	29.8	13.0
	23.0	10.5	42.6	20.0
Total.....do.....	137.7	62.0	235.0	113.4
Mean acre yields.....do.....	4,832	2,176	8,246	3,979
Net increase.....do.....			3,414	1,803
Increase.....per cent.....			70.7	82.9
Odds.....			4,201:1	3,332:1

TABLE 3.—*Acre and plot yields of snapped ears and of husked ears prime for canning, grown from Diplodia-infected Country Gentleman seed corn, untreated or treated with S. F. A. 225 dust, as planted in six replications in 1926, at Arlington Experiment Farm, Rosslyn, Va.—Continued*

## SUMMARY OF YIELD DATA

Series	Date of planting, 1926	Acre yield of prime corn (pounds)		Increase from treatment		Odds
		Untreated seed	Treated seed	Pounds per acre	Per cent	
No. 1.....	Apr. 6	1,260	2,853	1,593	126.4	9,999:1
No. 2.....	Apr. 14	2,046	3,239	1,193	58.3	285:1
No. 3.....	Apr. 22	2,319	3,867	1,548	66.8	1,683:1
No. 4.....	May 7	2,176	3,979	1,803	82.9	3,332:1
Mean.....		1,950.25	3,484.5	1,534.25	78.7	

Tables 2 and 3 and Figure 4 show that stands and yields were significantly increased as a result of dusting the seed with S. F. A. 225. Although the first planting was made under such unfavorable conditions that the stands and yields from untreated seed were very low, the total yield from the treated seed was practically the same as the total yield from treated seed in the more favored plantings. The yield of prime canning corn from treated seed in the first planting is higher than the yields of prime canning corn from untreated seed in any of the plantings. These data indicate that the practice of treating the seed may make feasible the practice of earlier planting of sweet corn, thereby solving certain farm-management problems and sometimes extending the canning season.

## EXPERIMENTS WITH COMMERCIAL SWEET-CORN SEED

Fifteen pounds each of two bulk lots of shelled seed of Country Gentleman were bought on the market in Washington, D. C. Both lots cost the same. By germinating 200 kernels from each, it was determined that lot 1 germinated 100 per cent and was nearly disease free, and that lot 2 germinated 90 per cent and 20 per cent of the kernels were *Diplodia* infected.

A seed-treatment experiment with these two lots of seed was conducted in the field near Bloomington, Ill. The 9 treatment materials were as follows: S. F. A. 225 dust, Semesan 13 UG dust, Semesan Jr. dust, Bayer dust, Du Pont 12 Bel dust, Abavit B dust, Du Pont 35 dust, Semesan, 0.5 per cent for a one and one-half hour soak, and Uspulun, 0.5 per cent for a one and one-half hour soak. The dusts were applied at the rate of 2 ounces per bushel and shaken with the seed in a closed container for 10 minutes. The plots were planted in rows of 22 hills each at the rate of 3 kernels per hill in hills spaced 42 inches each way. Since there were 6 replications of each treatment and 12 replications without treatment, 132 plots were required for the experiment. The yield data were obtained by the same methods used in obtaining the data presented in Table 3. The yields of prime canning corn are given in Table 4 and presented graphically in Figure 5.

TABLE 4.—Mean acre yields (average of six replications) of Country Gentleman sweet corn, prime for canning, with husks removed, grown from two lots of commercial seed, untreated or treated with each of several seed-treatment materials, respectively, as planted near Bloomington, Ill., in 1926

Seed treatment	Acre yield of prime corn with husks removed (pounds)		Increase in acre yield following treatment		Odds
	Untreated seed	Treated seed	Pounds	Per cent	
Lot 1, better seed					
Dusts.					
Semesan Jr.....	1, 972.5	2, 344.4	371.9	18.9	11:1
Bayer dust.....	2, 344.4	2, 473.7	129.3	5.5	3:1
Du Pont 12 Bel.....	2, 344.4	2, 392.9	48.5	2.1	2:1
Du Pont 35.....	2, 215.0	2, 328.2	113.2	5.1	3:1
S. F. A. 225.....	1, 972.4	2, 231.1	258.7	13.1	13:1
Semesan 13 UG.....	1, 972.4	2, 279.6	307.2	15.6	19:1
Abavit B.....	2, 215.0	2, 312.0	97.0	4.4	2:1
Soaks.					
Semesan.....	1, 972.5	2, 150.3	177.8	9.0	5:1
Uspulun.....	1, 972.5	2, 409.0	436.5	22.1	28:1
Mean.....	2, 109.0	2, 324.6	215.6	10.2	-----
Lot 2, poorer seed					
Dusts:					
Semesan Jr.....	1, 390.4	2, 037.1	646.7	46.5	1, 193:1
Bayer dust.....	1, 293.4	1, 616.8	323.4	25.0	21:1
Du Pont 12 Bel.....	1, 293.4	1, 681.4	388.0	30.0	55:1
Du Pont 35.....	1, 293.4	1, 600.6	307.2	23.8	14:1
S. F. A. 225.....	1, 390.4	1, 681.4	291.0	20.9	62:1
Semesan 13 UG.....	1, 390.4	1, 923.9	533.5	38.4	339:1
Abavit B.....	1, 293.4	1, 665.3	371.9	28.8	18:1
Soaks:					
Semesan.....	1, 196.4	1, 746.1	549.7	45.9	28:1
Uspulun.....	1, 196.4	1, 455.1	258.7	21.6	6:1
Mean.....	1, 304.2	1, 712.0	407.8	31.3	-----

As shown in Table 4 and Figure 5, certain seed treatments caused significant increases in yield. However, they were decidedly more beneficial to the poor commercial seed (lot 2) than to the good commercial seed (lot 1). The dust treatments were more consistent in their beneficial results than the liquid treatments, as judged by the odds obtained.

#### DISCUSSION

The results of experiments herein reported show that *Diplodia* seedling blight of sweet corn can be largely prevented by dust or liquid treatment of the seed with any one of several compounds, without injury even to the best seed. Conditions unfavorable for plant growth at planting time seem to increase the relative benefits from seed treatments. There are indications that dust-treated sweet-corn seed can remain longer in cold soils without injury than untreated seed. Hence, satisfactory stands may be obtained from dust-treated seed in earlier plantings than can be obtained from untreated seed. Dust seed treatments for the control of *Diplodia* seedling blight of sweet corn are as satisfactory as liquid treatments and possess certain advantages. They are easily applied and obviate the risk of injury to germination involved in wetting and drying the seed. Dust treatments can be made long in advance of planting time without injury to the seed, and tend to prevent insect infestation while such treated seed is stored. These features of the dust

method especially recommend it for use at central treating plants and by commercial firms handling the seed in quantity.

With sweet corn, as with dent corn, it is highly desirable to determine to what extent seed stock is infected with *Diplodia* and *Gibberella*, and to separate out the nearly disease-free seed in a manner now extensively used for dent corn. As with dent corn, nearly disease-free seed is not benefited by seed treatments, while second-grade seed usually is greatly benefited by the proper seed treatments.

### SWEET CORN PRIME FOR CANNING

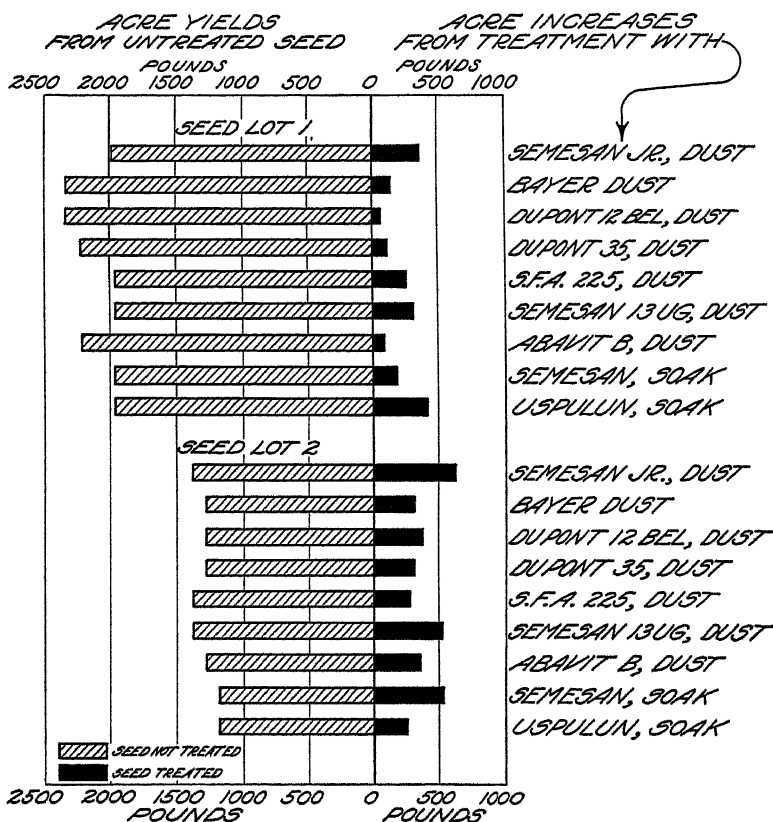


FIG. 5.—Acre yields of husked ears of Country Gentleman sweet corn, prime for canning, grown from treated and untreated seed of two commercial lots. (Data from Table 4)

The diseases caused by *Diplodia* and *Gibberella* are so important on sweet-corn seed that some commercial canners follow the practice of making ear germination tests of their sweet-corn seed stock and, on the basis of such tests, separate their seed into three lots, as follows:

No. 1. Nearly disease-free seed.

No. 2. Seed that germinates well, but carries some infection with *Diplodia*, *Gibberella*, and other similar organisms.

No. 3. Badly diseased seed.

The No. 1 seed is planted without seed treatment, the No. 2 seed is treated before planting, and the No. 3 seed is discarded or marketed, if any market can be found for it.

## SUMMARY

Satisfactory preliminary experiments to determine the relative merits of seed-treatment materials can be conducted in a greenhouse, if seed of sweet corn of good germination, highly infected with *Diplodia zeae*, is available.

Yields of prime canning corn from *Diplodia*-infected Country Gentleman seed treated with an organic-mercury dust were 126.4 per cent greater than yields from similar untreated seed when the planting was made in cold, moderately moist soil; 58.3 and 66.8 per cent greater when plantings were made in moderately cold, dry soil; and 82.9 per cent greater when plantings were made in warm, moderately wet soil.

Of two commercial lots of Country Gentleman seed, treatments were decidedly more beneficial to the corn grown from the poorer seed lot. Following treatment of the seed, the average increase in yield from the good lot was 10.2 per cent and from the poor lot 31.3 per cent over that from the untreated seed.

Dust treatments are more consistent in their beneficial effects, are more easily applied, and involve less risk of injury to the seed than liquid treatments.



# THE APPLE CURCULIO AND ITS CONTROL BY HOGS<sup>1</sup>

By B. B. FULTON, *Iowa State College*

## INTRODUCTION

The importance of the apple curculio (*Tachypterus quadrigibbus* Say) has usually been overshadowed by the emphasis given to the plum curculio (*Conotrachelus nenuphar* Herbst). While the latter does not thrive well under the conditions of modern orchard practice, the former seems to be affected very little if at all by spraying, soil cultivation, and pruning.

The destructiveness of the apple curculio in Iowa was first called to the writer's attention by R. M. Clark, manager of the Apple Grove Orchards, south of Mitchellville, Iowa. The plum curculio had been reduced to negligible numbers in this orchard but the manager estimated the loss caused by the apple curculio as at least a thousand dollars a year. This loss was due in part to the added cost of sorting out the unmarketable injured fruit.

The purpose of this paper is to report new observations on the seasonal history and habits of the apple curculio and to describe an effective method of control which can be easily applied over much of the territory in which this insect has proved to be destructive. A description of the stages of its development and many other previously reported facts concerning its life history have been omitted.

## REVIEW OF LITERATURE

The first important publication on the apple curculio was by Riley (7).<sup>2</sup> He describes and figures the main life stages and describes the punctures made in the green fruit, and the scars on mature pears identified as the early work of the apple curculio. It is evident, however, that his information on the life history is not derived from observations in the orchard. He states that infested apples never fall and that pupation takes place in the apple while it is still on the tree. Gillette (5) gives an account of the insect's work on apple and describes its oviposition habits. Crandall (4) has made the most complete study of the life history of the apple curculio in connection with his work on the plum curculio. In one respect his observations do not agree with those recorded here. He states that his new generation of beetles fed on the fruit to a very limited extent. Moreover, he describes and figures a type of injury which he ascribes entirely to the plum curculio, but which, in the opinion of the present writer, is more typical of that caused by the late summer feeding of the apple curculio.

The apple curculio has been mentioned as a pest of the apple in a number of widely separated localities. Its injury seems to be most severe in Missouri, Kansas, Illinois, Iowa, Maine, and the Province

<sup>1</sup> Received for publication Dec. 2, 1927; issued April, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 261.

of Quebec. Brooks (1) lists the following fruits as recorded hosts: Hawthorn, wild crab, haw, wild cherry, quince, pear, and apple. Petch (6) states that it attacks pear, plum, wild crab, cherry, and hawthorn. Watson (8) collected it on cotton in Florida. In northern Colorado it has been reported by List<sup>3</sup> as injuring 100 per cent of the sour cherries in certain orchards.

Control measures recommended for the apple curculio have usually been those found effective against the plum curculio. Such advice seems to be based on the similarity of habits of the two insects rather than on experimental data. Riley (7) predicts that the apple curculio can never be controlled by the tree-jarring method and says that the only real remedy is the destruction of infested fruit. Gillette (5) suggests arsenical sprays for ordinary infestations, supplemented when severe by jarring and the destruction of windfalls by means of hogs or sheep. Brooks (1) recommends the destruction of native host plants, jarring, and arsenical sprays, but says that the last will not prove as effective as against the plum curculio. Crandall's (4) discussion of control measures is concerned primarily with the plum curculio. Petch (6) states that arsenical sprays and dusts will give good results if applied at the right time and that lime sulphur and sulphur act as deterrents. Two popular reports of the control experiments recorded here have been published by R. M. Clark (2, 3) manager of the orchard where the work was conducted.

#### NATURE OF THE INJURY

The injury to fruit caused by the apple curculio may be locally severe, but it is not universally present in apple orchards, due in part no doubt to the strange reluctance of the insect to feed on apples of many of the common varieties. In many cases the cause of the injury is not known to the orchardist, or is thought to be the work of the plum curculio.

At the time the beetles are feeding very little injury will be noticed on the surface of the fruit. A few small holes like needle punctures may be observed, or the holes may be concealed by small black pellets of excrement. (Fig. 1, *e, f*.) If the holes are cut into toward the center of the apple deeper cavities will be found, which are of two types, large oval cavities and slender cavities more or less the shape of the insect's beak. The former are made for the dual purpose of obtaining food and holding an egg; the latter are feeding punctures only and are made largely by the males. (Fig. 1, *g-j*.)

The effect of such punctures is more apparent after considerable growth of the fruit has taken place. The cavity becomes almost completely obliterated within a short time by the rapid growth of the internal tissues of the young apple. This usually results in the destruction of the egg or young grub if the apple remains on the tree. The cavity is closed by the ingrowth of tissue from the sides and bottom, so that a longitudinal section often has the form of an inverted Y. (Fig. 2.) The cavity leaves a lasting imprint upon the growing fruit which becomes evident by the presence of a hardened area extending from the surface toward the core. Growth is ~~inhibited~~ at that point, and as the apple grows a funnel-shaped pit usually develops. (Fig. 1, *o*.)

<sup>3</sup> List, G. M. A NEW CHERRY PEST. Rocky Mt. Conf. Ent. Rpt. 4: 5. 1926. [Mimeographed.]



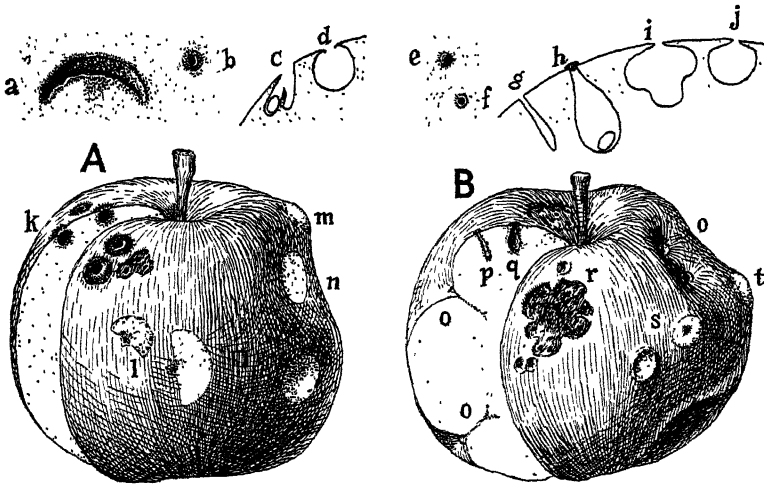


FIG. 1.—A, Injury to apple caused by plum curculio; B, injury to apple caused by apple curculio; a-j, recent injury on young fruit,  $\times 3$ ; k-l, recent injury on mature fruit and scars resulting from early injury; a, crescent cut or egg puncture, surface view; b, feeding puncture; c, d, sections of egg and feeding punctures; e, egg puncture plugged with excrement; f, same, plug removed; g, section of feeding puncture; h, section of egg puncture; i, j, unusual types of punctures; k, late summer-feeding punctures, enlarged by drying or rotting; l, scars resulting from crescent cuts; m, n, protruding and sunken scars from early feeding punctures; o, pits resulting from egg punctures; p, q, late summer-feeding punctures; r, patch of late summer-feeding punctures resulting in collapse of underlying tissues; s, t, craterlike and protruding scars resulting from early punctures

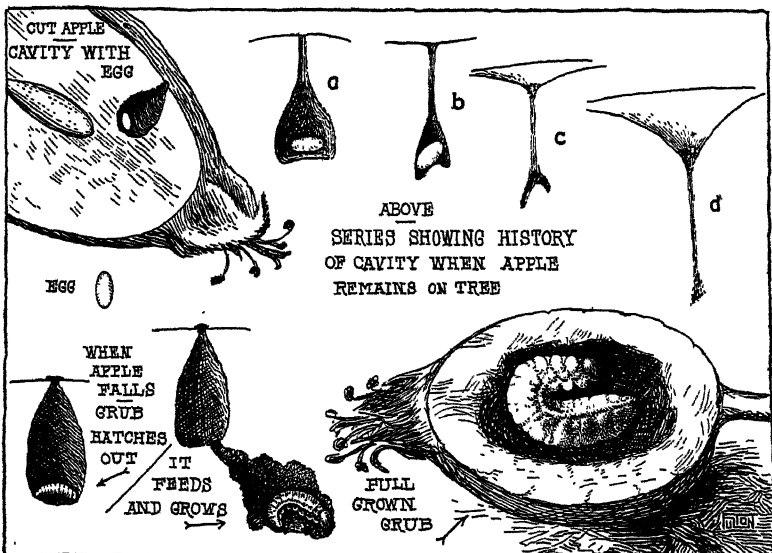


FIG. 2.—Cut apples and sections showing development of apple curculio grub in fallen apples, and history of egg cavities in growing apples

Under some conditions not fully understood the injury may develop as a protruding scar in the form of a rounded bump or a crater. (Fig. 1, *s, t*.) Usually an apple which has been affected at all receives a rather large number of punctures, with the result that as it develops it becomes very much deformed and is entirely worthless as a marketable fruit.

An entirely different type of injury results from the feeding of the new generation of beetles which appear in midsummer. It may be found from the middle of July on. The feeding cavities are like those made in the young apples, but they are not closed over by the growth of the apple. Instead, the portion of the skin undermined

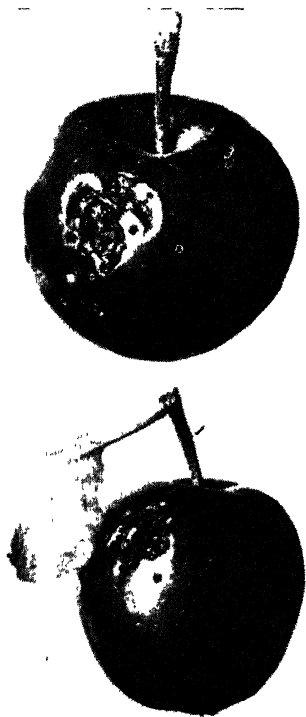
dries out, enlarging the opening. If the fruit is still quite green the inner part becomes a hardened scar lining the cavity. If the fruit is nearer maturity the pulp surrounding the cavity tends to dry out and shrink, causing the cavity to enlarge further. Usually a number of holes are located close together near the stem or calyx or under a sheltering leaf. In such cases the whole included area of skin dries and turns brown and the material underneath collapses as a result of the drying, so that no distinct cavities are apparent. All that one sees is a sunken and perforated brown patch on the side of the apple. (Fig. 1, *r*, and fig. 3.) The skin may shrivel and crack open, exposing the dried and discolored pulp. This type of injury has been ascribed to plum curculio, but punctures made by this insect in its late feeding are shallower and usually more scattered.

The work of the apple curculio can be distinguished from that of the plum curculio by the greater depth of the cavities formed. In

FIG. 3.—Punctures, concentrated under a protecting leaf, made by late-feeding apple curculio

the young fruit while the injuries are still recent the difference is very apparent. The plum curculio's beak is much shorter and broader than that of the apple curculio and the cavity formed is round close to the surface and the hole through the skin is rather large. (Fig. 1, *b, d*.) The egg punctures of the plum curculio are very easily recognized on account of the crescent-shaped cut along one side of the egg puncture proper. (Fig. 1, *a, c*.)

In the mature apples the plum curculio injuries are more apt to develop as spreading scars and usually lack the hardened internal scar tissue extending toward the core. The feeding punctures become rounded scars and the crescent cuts change to semicircles or half



moons. (Fig. 1, l.) These scars often occur in depressions but they are seldom deep funnel-shaped pits. In the case of apple curculio injury the pits rarely have more than a small scar at the bottom.

#### SEASONAL HISTORY

The apple curculio has but one generation a year and passes most of its life in the adult or beetle stage. It comes out of hibernation in the spring and begins feeding on the young apples just after they have set. Egg laying begins while the apples are still quite small. Most of the eggs are deposited before the June drop is complete, but a few beetles continue laying until the apples are nearly half grown. The egg-laying period covers a month or more. Each female deposits a few eggs a day, averaging 60 to 70 during the season.

Normally the apple must drop if the life history of the insect is to be completed. The entire larval and pupal stages are spent within

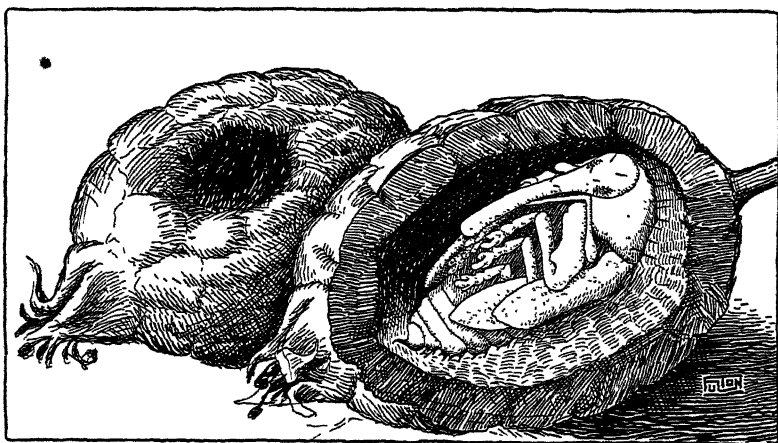


FIG. 4.—Fallen apple from which an adult apple curculio has escaped, and cut apple showing pupa

the dropped apple (fig. 4), which may become dried and mummified before the beetle emerges. Young living larvae were found in some apples picked off the trees in 1926, but none were found in apples picked in 1925. No large larvae have ever been found in growing apples. Evidently the insect is not completely adapted to the cultivated apple and is dependent on the June drop for survival.

A comparison of the percentage of injured fruit on the trees with that on the ground would indicate that the work of the apple curculio has little if any influence on the dropping. Dropped apples collected at several times during 1926 under one group of trees showed an infestation of 37 per cent in a total of 1,073. Counts of injured fruit on the same group of trees taken after the egg-laying season showed 39 per cent injured in a total of 703 apples.

The collecting of beetles and immature stages of the apple curculio has brought out facts concerning the seasonal history which corroborate the results obtained in previous life-history work and which are important in the application of control measures. The adults were found to be present throughout the year but became scarce

early in July because of the dying off of the old generation before many of their offspring had matured. A few of the old generation continued to live and deposited their eggs later in July. In the insectary the last egg was found on July 2 in 1925 and on July 7 in 1926. Eggs were probably deposited in the orchard subsequent to the latter date, for six larvae were collected in fallen apples on August 10, 1926. The transverse diameter of these apples (Ben Davis) was 20, 30, 30, 30, 35, and 38 mm. These larvae were probably from the last eggs deposited that season. Since the apples probably fell soon after the eggs were deposited, their size furnishes an indication of the limits of the egg-laying period. In Crandall's experiments eggs were deposited from May 23 to July 22, a period of over 60 days.

The number of beetles of the new generation leaving the fruit before July 1 must be negligible. The first beetle to be reared to maturity appeared July 3. After the first week in July the beetles began to mature in rather large numbers. By the middle of July 86 per cent had matured from the first dropped apples in 1925 and 100 per cent in 1926. (Table 1.)

TABLE 1.—Number of dropped and picked apples infested by the apple curculio, and number of curculios in the egg, larva, pupa, and adult stages on different dates in the summers of 1925 and 1926

Lot No.	Date collected	Date examined	Dropped or picked apples	Apples infested		Curculios in different stages							
				Number	Per cent	Egg		Larva		Pupa		Adult	
						Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1	June 17, 1925	June 18 <sup>1</sup>	Dropped			3	10	13	81				
	do	July 2 <sup>1</sup>	do					42	76	13	24		
	do	July 14 <sup>2</sup>	do							8	14	51	86
2	do	June 18 <sup>1</sup>	Picked			12	92	1	8				
	do	July 14 <sup>2</sup>	do					3	17	9	50	6	33
3	June 15, 1926	June 16 <sup>1</sup>	Dropped			2	10	18	90				
	do	June 30 <sup>1</sup>	do	487	33.0			52	32	110	68		
	do	July 13 <sup>2</sup>	do										100
4	July 14, 1926	July 15 <sup>2</sup>	Small early drops	205	39.0			10	15	33	52	21	33
5	do	do	Medium-sized drops	281	46.0			45	36	79	64		
6	do	do	Recent drops	100	24.0			24	100				
7	Aug. 9, 1926	Aug. 10 <sup>2</sup>	Dropped	205	6.8			7	54	4	31	2	15
8	June 15, 1926	June 16 <sup>1</sup>	Picked			26	58	19	42				
	do	July 1 <sup>2</sup>	do					52	84	10	16		

<sup>1</sup> Part.

<sup>2</sup> Remainder.

On account of the extended egg-laying period it is of considerable interest to find out the probable proportion of eggs deposited at different times as the season advances. The heaviest drop of apples comes during a period of a few weeks in June, several weeks after the blossoming period. The proportion of fruit falling during the June drop is quite large except when very few blossoms have set. In 1926 the set of fruit was average or better and the June drop was normal. The apples in lots of 3, 4, 5, 6, and 7 (Table 1) were collected from the same rows of trees and show by the number infested something of the amount of egg laying at the time they fell. Lot 3, which was 33 per cent infested, included the first June drop prior to June

15. Lots 4, 5, and 6 were all collected July 14 and were segregated by size. Lot 4 included the small withered apples which probably fell soon after the middle of June; lot 5 included larger withered apples which probably fell in late June or early July; lot 6 contained only larger fruit which could not have been off the trees much more than a week. The degree of infestation in the three lots was 39 per cent, 46 per cent, and 24 per cent. Lot 7, collected August 9, contained apples which appeared to have fallen so recently that the curculios could not have emerged from them. They were only 6.8 per cent infested.

The percentage of infested apples in lot 6 is still quite high, but the number of apples that dropped in July must have been considerably reduced. The number dropping between the middle of July and August 9 must certainly have been small compared to the June drop. Of the set fruit which falls before the approach of maturity it is probably safe to say that three-fourths drops before the 1st of July in a normal year. Since the degree of infestation is greater before that date it seems probable that 80 per cent of the whole brood of apple curculios are on the ground by July 1. All but a small fraction of the remainder probably appear before the middle of July.

#### HABITS OF THE ADULTS

On account of the peculiar shape and protective coloration of the beetles, they are extremely difficult to find until the eye has become trained to detect them. They have a habit of posing with beak elevated when anything approaches them (fig. 5), and while standing perfectly motionless they are easily mistaken for a loose bud scale or a cluster of dried petals. If a moving object comes close to them they will drop. The readiness with which they crawl away or take to flight would make a tree-jarring method of control as sometimes used against the plum curculio impractical for this insect.

The adults feed mainly on the fruit, consuming large quantities, especially the females when excavating the egg cavities. To a certain extent the beetles will feed on leaves, making small holes about one-thirty-second of an inch or less. They feed on the cambium of green spurs in much the same way as on the fruit, by enlarging the cavities beneath the surface. This type of feeding was observed in the case of caged insects and its effect can be noted on the orchard trees.

#### HIBERNATION

An effort was made to find the adult apple curculios in winter to determine where their favorite hibernation quarters are. All search failed of results. Another attempt to solve this problem was made by confining beetles in a large cage under an orchard tree. On August 11, 1926, 80 adult curculios were placed in a cage which contained an old partly decayed apple stump with loose bark, a piece of burlap, dead leaves, matted grass, and a number of apples.

On October 25 the cage was examined and a careful search was made under loose bark, under leaves, and in the upper soil. Fifteen living and two dead curculios were recovered. All were found close to the surface of the ground under matted grass, and most of them were near the door where they had been put in. Apparently they

had not moved about much after August 11. What became of the other 63 beetles is difficult to say, but most of them were probably overlooked in the search. The cage was large, and, as stated before, this insect is difficult to find. None of the apples in the cage had been fed upon.

#### RESISTANCE OF LARVAE AND PUPAE TO HEAT

One of the control measures usually recommended for the apple curculio is to expose the fallen fruit to sunlight by raking it between the rows or by pruning the trees to let in more sunlight. It was thought advisable to test the effectiveness of sunlight as a killing agent.

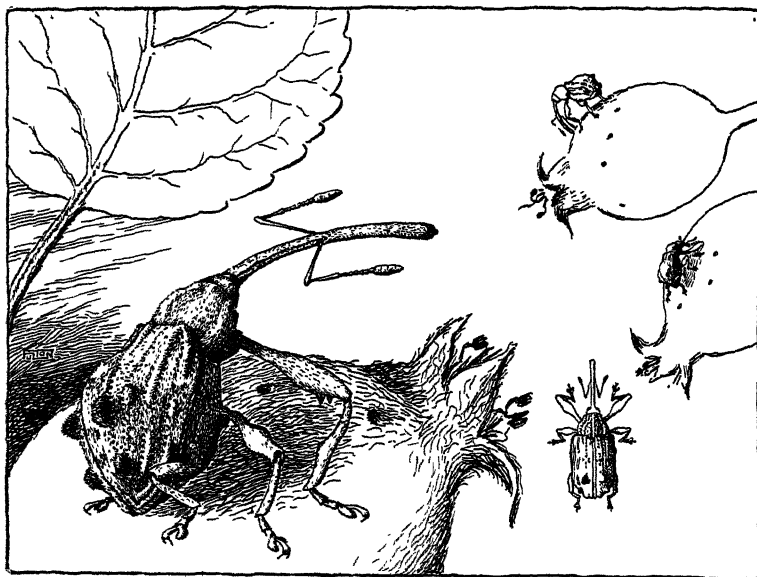


FIG. 5.—Adult apple curculio

On a very hot day a number of infested dropped apples were exposed to the sun by spreading them out in a single layer in a flat pan. The temperature under a tree at the time ranged from  $103^{\circ}$  to  $103\frac{1}{2}^{\circ}$  F. On the surface of the ground in the sun the temperature was  $135^{\circ}$ ; the surface of the pan was  $125^{\circ}$ – $126^{\circ}$ , and when the bulb was placed within the cavity of one of the apples the thermometer registered  $118^{\circ}$  to  $120^{\circ}$ . The last was probably about the temperature to which the insects were exposed.

After 15 minutes' exposure to the sun, six of the apples were removed and opened. Four larvae were found dead and two alive. After a half hour's exposure another lot was removed and 10 larvae and 5 pupae were found dead. The remaining 9 larvae and 5 pupae were exposed for an hour and all died. As a check, a number of apples from the same lot were examined without exposure to the sun. One dead and apparently parasitized larvae was found among 17 living larvae and 2 pupae.

From this experiment it seems improbable that the insects could live long if the apples were removed from under the trees and exposed to direct sunlight on cultivated ground. Cultivation after the apples have dropped would tend to protect the larvae, however, by shielding them from direct sunlight. Experiments with buried apples showed that the beetles could mature and come out from 2½ inches of loam soil, both loose dry soil and soil that had been water-soaked and dried. Some curculios escaped from 6 inches of soil but ordinary disking probably would not bury apples more than 2 or three inches.

#### PARASITISM

Parasitism could not have had any marked effect on the degree of infestation of the orchards at Mitchellville. In 1925 only one parasite was discovered in all the material examined. In 1926 the parasites had apparently increased to some extent. In one lot of 235 curculios in various stages, 17 (7.2 per cent) were parasitized. The parasite was a small chalcid fly which was identified by A. B. Gahan as a species of *Eurytoma*, probably undescribed. The larvae feed externally on the curculio grub. Usually only a withered carcass is found with a full-grown parasite larva. The first adult parasite appeared on July 20, 1926.

#### CONTROL EXPERIMENTS

Experiments on the control of the apple curculio were all carried on at the Apple Grove Orchards south of Mitchellville, Iowa, or with insects obtained from there. The three orchard plots, separated by intervening field and woods, are described as follows: (1) three acres, 90 per cent Ben Davis variety, woods on three sides; (2) 17 acres, 40 per cent Ben Davis, woods on three sides; (3) main block of 24 acres, 30 per cent Ben Davis, with a spur of 3 additional acres on the southwest corner, 70 per cent Ben Davis. The main block is surrounded by open fields but the spur is directly across the road from a wooded pasture. The apple curculio became severe in orchard No. 2 near the timber about 1915. It spread slowly and by 1925 was most severe in orchard No. 1 and the 3-acre spur of No. 3. The main block of No. 3 remained relatively free but was showing an increase in infestation. The location of the original points of attack would indicate that the curculio came into the orchards from the wood lots, or found more favorable conditions near the woods. The increase in infestation was not due to an overflow of curculios breeding in wild crab apples or hawthorns, however, for such trees had been removed from all woods, except on the north side of orchard No. 1, where the land was under different ownership.

In these orchards the most extensive injury has been done to the Ben Davis variety. A few bearing Delicious in orchard No. 2 have been about as severely injured. Grimes Golden and Willow Twig have been injured to some extent, and Jonathan, Stayman, and Wine-sap have been slightly injured. Northwestern Greening remained free from injury even when adjacent to the worst infested trees.

Attempts to control the apple curculio by applying an extra lead-arsenate spray between the "calyx" and "first-cover" sprays were unsuccessful. The orchards were disked until mid-July, trees were pruned for more sunlight, adjacent fence rows were cleaned up, and

woodland west and north of orchards 3 and 4 were thinned out and pastured. When hogs were available they were turned into the orchard, but this was done only in the spring. These measures resulted in the practically complete elimination of the plum curculio in the orchards but had no lasting effect on the apple curculio. After nine years the latter was more destructive than ever.

In the spring of 1925 an experiment was begun in which hogs were used more intensively. During April and May the orchards were cultivated with a 14-foot extension disk to eliminate as much grass as possible. Pigs weighing from 65 to 90 pounds removed most of the remaining grass under the trees by early June. They then began to feed on the fallen green apples. Until the June drops were well cleaned up in July almost no grain was given them. The number of pigs and the period of pasturage are shown in Table 2 for each orchard.

TABLE 2.—*Number of pigs and period of pasturage in the different orchards in 1925 and 1926*

Orchard No.	Number of acres fenced	Number of pigs pastured in 1925	Period of pasturage in 1925	Number of pigs pastured in 1926	Period of pasturage in 1926
1.....	3	15	June 25-Aug. 30.....	0	
2.....	17	50	June 6-July 20.....	45	Mar. 1-June 20.
3.....	3	15	Apr. 1-June 25 and 5 or 6 days in July.....	45	June 20-July 20.
	24				

In considering the effect of control measures one must bear in mind that the use of pigs is directed against the new generation of curculios and has practically no effect on the early injury to the fruit. To some extent, then, the amount of injury in the orchard in 1925 could be considered a check on the control obtained for the following year.

The dropped apples were cleaned up very thoroughly in the 3-acre spur of orchard No. 3. Counts of injured and uninjured fruit in this block were made on August 25, 1925. Apples on the lower branches only were counted. On 13 trees there were 1,751 apples. Of these, 1,232 showed injury and 519 showed none; that is, 70 per cent of the fruit was injured. The injury to fruit on individual trees ranged from 40 to 95 per cent.

A very small portion of the injury was caused by the late feeding of the new generation of beetles. This late type of injury had heretofore been quite important in the orchard and added considerably to the total loss.

On July 14, 1926, counts were made in the same part of the orchard. A total of 5,246 apples were examined and of these only 93 (1.77 per cent) showed any injury by apple curculio. The fruit injury of individual trees ranged from 0 to 4.7 per cent.

Orchard No. 1, which had also been thoroughly pastured by hogs in 1925, yielded 500 bushels of apples in 1926, the first paying crop that had been harvested from that block for six years. No counts of injured fruit had been taken in this orchard, but the improvement in the crop was as marked as in No. 3. In 1926 dropped apples containing larvae were scarce, whereas in 1925 an abundance of larvae were obtained by picking up dropped apples at random.



In 1925 orchard No. 2 was only a little less infested than No. 1 and No. 3. A small plot in the southeast corner, separated from the remainder by a gully, was very thoroughly freed from drops in 1925 due to the presence of a wallow in the gully which kept the pigs in that part of the orchard. In 1926 these trees were practically free from curculio injury.

Farther back in the orchard the drops were not so well picked up, and in 1926 the injury in this part was estimated as a check on the figures taken in orchard 3, where the clean-up had been thorough. On August 9, 1926, counts made of injured and uninjured fruit on five trees gave a total of 278 injured and 425 uninjured, or 39 per cent injured. The percentages of injury on the five trees were 16, 28, 32, 43, and 68. Thirty-seven per cent of the dropped apples collected in the same part of the orchard during the season were infested.

The experiments with pasturing pigs were successful from a business standpoint. A cost account kept for the two years showed that this method of control was more than economical, for it actually netted a profit. In 1925 each pig returned a net profit of \$10 above cost and feed and in 1926 a net profit of \$7.65. These figures include the cost of vaccination but not the item of labor in handling or feeding the pigs, nor the cost of fencing, which in his case was small.

A few experiments were tried to determine the possibility of poisoning curculios with arsenical sprays and dusts. Five curculios were caged on a small branch sprayed with calcium arsenate at the strength of 1 pound to 50 gallons of water plus 3 pounds of lime. Five curculios were caged on a second branch sprayed with the same material with the addition of enough molasses to give a slightly brown color. None of the insects showed any sign of poisoning and continued to feed.

On June 5, 1925, 25 curculios were placed in each of three different wire cages over branches of a tree. One branch was thoroughly dusted with calcium arsenate after the cage was in place, so that some of the powder settled on parts of the cage. Another branch was sprayed with the same material at the rate of 1 pound of the poison to 50 gallons of water; the third was untreated. On June 9, the cages were examined but not opened. The one with the dusted branch had 17 dead curculios in the bottom, the one with the sprayed branch had 4 dead, and the check had 1 dead. On June 10 the first two cages were removed. In the cage with the dusted branch 20 curculios were dead and 3 alive, leaving 2 unaccounted for; in the cage with the sprayed branch 4 were dead and 21 alive; in the check cage only 1 was dead. Apples on the check and sprayed branches were riddled with punctures, while those on the dusted branch showed no signs of having been fed upon.

On June 12 the same experiment was repeated in the greenhouse. A small branch was sprayed with calcium arsenate, 1 gm. in 400 c. c. of water, of which about one-fourth was applied. Another branch of similar size was dusted, using 1 gm. of calcium arsenate applied with a small hand duster. Cages were fitted over the branches and 10 curculios were released in each before the sprayed branch was quite dry. On June 15 all curculios were alive and the fruit had many feeding punctures. From the fact that the insects lived so long after the treatment it was apparent that the poison had had little or no effect on them. The success of the dusting in the previous

experiment was possibly due to the large quantity used or to the deposit in the bottom of the cage where the curculios may have crawled about.

As a field experiment, a number of trees were dusted with calcium arsenate, using a rotary fan type of hand duster from a stepladder. On July 2, five days after treatment, the lower branches were shaken over a tarpaulin. The number of curculios collected from the dusted trees were 0, 1, 2, 4, 8, 2, 9, 0, 0, 3. All except one were alive. Only two untreated trees were shaken, yielding a catch of 2 and 1. A few weeks before, 20 or 30 curculios could have been collected easily from one tree. It seemed evident that the small number taken from the treated trees was not due to poisoning, but to natural dying off at this time. The new generation had not yet come out in quantity.

#### CONCLUSIONS AND RECOMMENDATIONS

The experiments with the use of poisons, supplemented by the observations of Clark in the orchard, show that there is little hope of controlling the apple curculio with arsenical poisons. Crandall (4) reached the same conclusion after feeding apple curculios on fruit treated with Paris green.

The experiment with the use of hogs has shown that five pigs per acre can, if properly handled, clean up the early dropped apples in an orchard and thus control the apple curculio. The critical time for such control, as shown by the seasonal history data, is from the middle of June until about the middle of July. Pigs weighing about 100 pounds are the best size for this purpose since they do not tramp down the low branches. They do not feed from the trees to any great extent if the apples are more than a foot above the ground.

The pigs prefer green apples to grass, and they can find the apples more readily if the orchard is cultivated before the middle of June. No cultivating should be done after the apples begin to drop.

Pigs should be encouraged to frequent parts of the orchard containing the varieties most subject to injury by the apple curculio. This can be done by throwing there whatever extra feed is necessary. The best results will be obtained if the pigs are kept on slightly short rations.

The greatest drawback to keeping pigs in the orchard is that they injure the trees by rooting or by rubbing against the trunks. When small pigs are used for only a month such injury is negligible. Pigs should not be oiled while they are in the orchard, on account of possible injury to the trees from oil rubbed into the bark.

It is sometimes difficult to obtain pigs at the time when the pasturing should be done. The fruit grower should therefore anticipate this need and buy them during the winter and spring whenever they can be obtained most economically.

#### SUMMARY

Both the early and late injuries to apples caused by the apple curculio show certain characteristics which distinguish them from similar injuries by the plum curculio.

Egg punctures formed by the apple curculio are closed up rapidly by the growth of the apple. Larvae survive and mature only in

apples which drop from the trees. There is no evidence to indicate that the presence of eggs or larvae causes dropping.

Probably 80 per cent or more of the total new brood of curculios likely to survive will be found on the ground in the egg, larval, or pupal stage by July 15. Adults of the new generation begin to emerge about the 1st of July.

Curculios in immature stages in the apples are killed by exposure to sunlight on bare ground.

Adults are able to emerge when apples are buried under a few inches of soil.

The apple curculio can not be readily controlled by arsenical sprays, orchard cultivation, or the destruction of wild host plants.

Dropped apples may be effectively destroyed and severe infestations of the apple curculio controlled by confining a sufficient number of hogs in the orchard during early summer.

#### LITERATURE CITED

- (1) BROOKS, F. E.  
1910. THREE SNOUT BEETLES THAT ATTACK APPLES. W. Va. Agr. Expt. Sta. Bul. 126, p. [105]-124, illus.
- (2) CLARK, R. M.  
[1926]. REPORT OF PROGRESS ON THE CONTROL OF THE APPLE CURCULIO. Iowa State Hort. Soc. Rpt. (1925) 60: 39-42.
- (3) ———  
[1927]. FURTHER REPORT ON THE CONTROL OF THE APPLE CURCULIO. Iowa State Hort. Soc. Rpt. (1926) 61: 33-35.
- (4) CRANDALL, C. S.  
1905. THE CURCULIO AND THE APPLE. Ill. Agr. Expt. Sta. Bul. 98, p. 467-560, illus.
- (5) GILLETTE, C. P.  
1890. THE APPLE CURCULIO. Iowa Agr. Expt. Sta. Bul. 11: 492-494, illus.
- (6) PETCH, C. E.  
1925. THE APPLE CURCULIO AND ITS CONTROL IN QUEBEC. Canada Dept. Agr. Ent. Branch Circ. 36, 4 p., illus.
- (7) RILEY, C. V.  
1871. THE APPLE CURCULIO. In Third Annual Report on the Noxious, Beneficial and other Insects of the State of Missouri, p. 29-35, illus. Jefferson City, Mo.
- (8) WATSON, J. R.  
1921. NOTES ON SOME FLORIDA WEEVILS. Fla. Ent. 4: [33]-35.



# THE RELATIVE UTILIZATION OF DIFFERENT CALCIUM COMPOUNDS BY HENS IN THE PRODUCTION OF EGGS<sup>1</sup>

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## INTRODUCTION

In another publication<sup>2</sup> the authors presented evidence to show that calcium in rock phosphate, when fed to hens on a grain-bran-tankage ration, is not utilized in the formation of eggshell; at least, not to the same extent as calcium in the form of carbonate. The experiment described herein was planned to determine to what extent the hen can utilize for egg production the calcium in calcium lactate, calcium chloride, calcium sulphate, and pure tricalcium phosphate, as compared with that in calcium carbonate.

## EXPERIMENTAL PROCEDURE

On January 1, 1926, 40 one-year-old White Leghorn hens from the same parent stock were placed in four similar houses. These houses were so constructed that large windows faced the south and east, allowing the free entrance of direct sunlight for several hours each day. The hens received a ration consisting of wheat and yellow corn, and skim milk was always at their disposal. Green food, such as cabbage and lettuce leaves, was given twice a week, and the straw litter covering the floor was changed frequently. From January 1 to March 11, 1926, no calcium compound was added to the ration given to the four lots.

A trap-nest record of the eggs was kept throughout the experiment. The eggs laid by each hen each week were weighed together at the end of the week. The shells were then carefully removed and dried for 12 hours at 100° C. in an electric oven, and after cooling they were weighed. The difference between the average weight of the eggs for a given period and the average weight of the shells for the same period is taken as the average weight of the contents.

On March 11, 1926, the hens were weighed individually and divided into 4 lots of 10 each, designated lots 1, 2, 3, and 4. The lots were so divided as to equalize their weights and egg-laying ability. The average weights of the hens in lots 1, 2, 3, and 4 were 1,825, 1,813, 1,826, and 1,825 gm., respectively, and the average egg records for the pullet year were 165, 162, 169, and 164 eggs per hen, respectively.

For the sake of clarity, each lot will be discussed separately. It is to be remembered that all the hens had gone for 70 days without a calcium supplement to their diet, before the calcium compound to be tried was added.

<sup>1</sup> Received for publication Nov. 21, 1927; issued April, 1928. Published with the approval of the director of the Kentucky Agricultural Experiment Station.

<sup>2</sup> BUCKNER, G. D., MARTIN, J. H., and PETER, A. M. CALCIUM METABOLISM IN THE LAYING HEN. Ky. Agr. Expt. Sta. Bul. 250, p. 329-367. 1923.

## EXPERIMENTAL DATA

## LOT 1

Beginning March 12, 1926, a very high grade limestone (96.3 per cent  $\text{CaCO}_3$ ) crushed to about one-fourth inch in size and screened free of dust was kept before the hens constantly until April 11, 1926. During this time the 10 hens consumed 2,680 gm. of the limestone, which is at the rate of 8.7 gm. per hen per day. On April 12 the limestone was replaced by rock gypsum crushed to about the same size as the limestone, screened, and freed from carbonates by treatment with hydrochloric acid and washing free of chlorides. This purified rock gypsum contained 94.6 per cent  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ . Between April 12 and July 18, 1926, the hens consumed 6,090 gm. of the purified gypsum, which was at the rate of approximately 6.3 gm. per hen per day, and it was greedily eaten.

TABLE 1.—*Number of eggs produced by hens of lot 1, kept on a grain-skim-milk ration fed without the addition of mineral or with the addition of calcium carbonate or calcium sulphate; together with the average weight of the eggs, shells, and contents*

Additions to basal ration	Number of days of feeding	Number of hens	Total number of eggs	Number of eggs per day per 100 hens	Average weight of 1 egg	Average weight of contents	Average weight of dry eggshell
No mineral added*					Gm.	Gm.	Gm.
Jan. 1-31.....	31	10.0	18	6	53.3	48.6	4.7
Feb. 1-Mar. 11.....	39	10.0	41	11	55.9	51.5	4.4
Calcium carbonate added:							
Mar. 12-Apr. 11.....	31	10.0	179	58	56.9	51.2	5.7
Calcium sulphate substituted for calcium carbonate:							
Apr. 12-30.....	19	10.0	124	65	57.1	52.3	4.8
May 1-17.....	17	10.0	102	60	53.4	49.2	4.2
May 18-June 14.....	28	10.0	104	37	50.5	46.4	4.1
June 15-July 18.....	34	9.5	26	8	51.8	47.8	4.0

\* 1 hen died July 2.

It will be seen in Table 1 that the average weight of the eggs for the 39 days previous to March 12, 1926, was 55.9 gm., that of the contents, 51.5 gm., and that of the dry shells, 4.4 gm. The addition of calcium carbonate to the ration did not increase the average weight of the contents of the eggs during the following 31 days, but the average weight of the dry eggshells was increased 1.3 gm. After the addition of the carbonate more than five times as many eggs per day were produced. Immediately after the calcium carbonate was replaced by calcium sulphate there was a slight increase in the daily egg production, but after May 18, when production should normally remain high, it diminished rapidly. There was also a lowering in the average weights of the contents and of the dry eggshells. These facts indicate that calcium carbonate is utilized to a much greater extent by the hen for the production of eggs than is calcium sulphate. An examination of the droppings of the hens showed that large quantities of limestone and gypsum were being passed through the hens unchanged while each was being added to the ration.

## LOT 2

From March 12 to April 11, 1926, during which time they had free access to the prepared gypsum described under lot 1, the 10 hens consumed 1,300 gm. of that mineral, which is at the rate of 4.2 gm. per hen per day. Soon after the gypsum was added to the diet, the droppings became soft and slightly watery and this condition continued in varying degree. The number of eggs laid by the hens was daily becoming less and the general condition of the birds was not good; so the gypsum was replaced by limestone on April 12. The hens consumed 4,105 gm. of limestone between that date and July 18. This is at the rate of approximately 4.2 gm. of limestone per hen per day.

TABLE 2.—*Number of eggs produced by hens of lot 2, kept on a grain-skim-milk ration fed without the addition of mineral or with the addition of calcium sulphate or calcium carbonate; together with the average weight of the eggs, shells, and contents*

Additions to basal ration	Number of days of feeding	Number of hens	Total number of eggs	Number of eggs per day per 100 hens	Average weight of 1 egg	Average weight of contents	Average weight of dry eggshell
No mineral added:					Gm.	Gm.	Gm.
Jan. 1-31.....	31	10	13	4	60.9	56.4	4.5
Feb 1-Mar. 11.....	39	10	48	12	57.8	53.6	4.2
Calcium sulphate added:							
Mar. 12-Apr. 11.....	31	10	70	23	53.9	49.4	4.5
Calcium carbonate substituted for calcium sulphate:							
Apr. 12-30.....	19	10	110	58	58.8	53.4	5.4
May 1-17.....	17	10	108	63	58.2	53.2	5.0
May 18-June 14.....	28	10	121	43	56.8	51.6	5.2
June 15-July 18.....	34	9	107	35	57.1	51.9	5.2

In Table 2 it will be seen that the egg production was nearly doubled following the addition of calcium sulphate to the ration of grain and skim milk. However, this increase was considerably less than the increase in egg production caused by the addition of calcium carbonate to the same ration in lot 1. The average weight of contents was 4.2 gm. less for the period the calcium sulphate was added to the diet than for the 39 days preceding, when no calcium supplement was used, whereas the average weight of the dry shells remained practically unchanged. When calcium carbonate was substituted for calcium sulphate, the average weights of the contents and dry shells were increased materially and the number of eggs laid was more than doubled. The decline in egg production following May 18, was slower when calcium carbonate was substituted for calcium sulphate than when the sulphate was substituted for the carbonate in lot 1.

## LOT 3

From March 12 to June 14, 1926, the hens received calcium lactate (98.2 per cent) daily in 200 gm. of a wet mash consisting of equal parts of bran, shorts, and corn meal, at the rate of 5 gm. of calcium lactate per hen per day for the first 20 days and 10 gm. per hen per day from then until June 14. When the calcium lactate mash was first given the hens, it was eaten greedily, but on May 18 it was

noticed that the hens were not eating it all and by June 14, it was refused entirely. On June 15 calcium carbonate (limestone) was substituted for the calcium lactate and the hens consumed 740 gm. during the next 34 days, which is at the rate of approximately 2.2 gm. per hen per day.

It is believed that the introduction of the wet mash, as such, into the diets of lots 3 and 4 could not have influenced egg production materially.<sup>3</sup>

TABLE 3.—*Number of eggs produced by hens of lot 3, kept on a grain-skim-milk ration fed without the addition of mineral or with the addition of calcium lactate or calcium carbonate; together with the average weight of the eggs, shells, and contents*

Additions to basal ration	Number of days of feeding	Number of hens	Total number of eggs	Number of eggs per day per 100 hens	Average weight of 1 egg	Average weight of contents	Average weight of dry eggshell
No mineral added:					Gm.	Gm.	Gm.
Jan. 1-31.....	31	10	16	5	53.9	50.1	3.8
Feb. 1-Mar. 11.....	39	10	48	12	54.1	49.9	4.2
Calcium lactate added:							
Mar. 12-Apr. 11.....	31	9	93	33	53.9	49.6	4.3
Apr. 12-30.....	19	9	89	52	54.6	49.7	4.9
May 1-17.....	17	9	79	52	54.3	49.6	4.7
May 18-June 14.....	28	9	81	32	53.4	48.9	4.5
Calcium carbonate substituted for calcium lactate: June 15-July 18.....	34	8	130	48	57.9	52.8	5.1

The addition of calcium lactate to the ration did not materially change the average weight of the contents (Table 3), but caused a slight increase in the average weight of the dry shells. There was, however, a marked increase in the number of eggs laid. When the hens began to refuse to eat the mash on May 18 there was a noticeable fall in egg production. When calcium carbonate was added, egg production was increased and the average weights of the contents and the dry eggshells were greater. It seems therefore that the calcium in calcium lactate can be utilized by the hen for egg production to the extent that it is consumed.

#### LOT 4

From March 12 to April 5, 1926, 200 gm. of a wet mash consisting of equal parts of bran, shorts, and corn meal, containing 4 gm. of calcium chloride (81.0 per cent) per hen was fed daily. The addition of calcium chloride caused some diarrhea, which was accompanied by loss of appetite for the mash. On April 5 the hens refused to eat any of the mash, and since egg production had not greatly increased, precipitated tricalcium phosphate (99.2 per cent) was substituted for the calcium chloride and was given at the rate of 10 gm. per hen per day in the same wet mash. The mash containing phosphate was eaten greedily and the amount supplied to the hens was consumed completely. Because most of the hens had stopped laying by June 14 limestone (calcium carbonate) was substituted for the tricalcium phosphate. The hens consumed 395 gm. of the limestone, which is at the rate of approximately 1.2 gm. per hen per day.

<sup>3</sup> MARTIN, J. H. SOURCES OF ANIMAL PROTEIN FOR LAYING HENS. Ky. Agr. Expt. Sta. Bul. 260, p. 99-132, illus. 1925.



TABLE 4.—Number of eggs produced by hens of lot 4, kept on a grain-skim-milk ration fed without the addition of mineral or with the addition of calcium chloride, tricalcium phosphate, or calcium carbonate; together with the average weight of the eggs, shells, and contents

Additions to basal ration	Number of days of feeding	Number of hens	Total number of eggs	Number of eggs per day per 100 hens	Average weight of 1 egg	Average weight of contents	Average weight of dry eggshell
No mineral added:					Gm.	Gm.	Gm.
Jan. 1-31.....	31	10	25	8	80.3	55.4	4.9
Feb. 1-Mar. 11.....	39	10	37	9	56.1	51.6	4.5
Calcium chloride added.							
Mar. 12-Apr. 5.....	25	9	45	20	55.1	51.0	4.1
Tricalcium phosphate substituted for calcium chloride:							
Apr. 6-30.....	25	9	59	26	52.9	48.5	4.4
May 1-17.....	17	8	38	28	53.9	49.1	4.8
May 18-June 14.....	28	8	51	23	51.9	47.2	4.7
Calcium carbonate substituted for tricalcium phosphate:							
June 15-July 18.....	34	7	88	37	54.6	49.5	5.1

The results in Table 4 show that the addition of calcium chloride was followed by a small increase in the number of eggs laid and a small lowering in the average weights of the contents and the dry shells. After the substitution of tricalcium phosphate there was some increase in egg production, a decrease in the average weight of the contents, and a slight increase in the average weight of the dry eggshells. When calcium carbonate was substituted for tricalcium phosphate there was an increase in egg production and a noticeable increase in the average weights of the contents and dry shells. This is significant because it occurred at a period (June 15 to July 18) when a decline in egg production and egg weights is to be expected.

In order to bring out more clearly the effects following the addition of the several calcium compounds to the basal ration, Tables 5 and 6 have been prepared. Here are shown the comparative production of eggshell and egg contents during the several feeding periods, taking as unity the average production of all the hens during the 39 days, February 1 to March 11, when they were not receiving added calcium. The figures were obtained by dividing the weight of shell or contents produced per 100 hens per day during a given period by the corresponding weight per 100 hens per day for the 39-day period when no calcium compound was added to the ration. The figures show strikingly the superiority of calcium carbonate to the other calcium compounds used. A temporary increase in production of egg contents following the substitution of calcium sulphate for calcium carbonate in lot 1 is apparent, but the production of shell did not increase correspondingly.

TABLE 5.—*Relative amounts of eggshell produced by hens kept on a grain-skim-milk ration with the addition of different compounds of calcium, the average production of the same hens during the preceding 39 days on the same basal ration without added calcium being taken as unity*

Days of consecutive feeding periods	Lot 1	Lot 2	Lot 3	Lot 4
31	Carbonate, 7.0	Sulphate, 2.2	Lactate, 3.0	Chloride,* 1.7
19	Sulphate, 6.6	Carbonate, 6.6	Lactate, 5.4	Phosphate,* 2.4
17	Sulphate, 5.3	Carbonate, 6.6	Lactate, 5.2	Phosphate, 2.8
28	Sulphate, 3.2	Carbonate, 4.7	Lactate, 3.0	Phosphate, 2.3
34	Sulphate, 0.7	Carbonate, 3.8	Carbonate, 5.2	Carbonate, 4.0

\* 25 days of feeding.

TABLE 6.—*Relative amounts of egg contents produced by hens kept on a grain-skim-milk ration with the addition of different compounds of calcium, the average production of the same hens during the preceding 39 days on the same basal ration without added calcium being taken as unity*

Days of consecutive feeding periods	Lot 1	Lot 2	Lot 3	Lot 4
31	Carbonate, 5.2	Sulphate, 2.0	Lactate, 2.9	Chloride,* 1.8
19	Sulphate, 6.0	Carbonate, 5.5	Lactate, 4.6	Phosphate,* 2.2
17	Sulphate, 5.2	Carbonate, 5.9	Lactate, 4.5	Phosphate, 2.4
28	Sulphate, 3.0	Carbonate, 3.9	Lactate, 2.8	Phosphate, 1.9
34	Sulphate, 0.7	Carbonate, 3.2	Carbonate, 4.5	Carbonate, 3.2

\* 25 days of feeding.

## SUMMARY

A study has been made of the comparative effectiveness of calcium carbonate, calcium sulphate, calcium lactate, precipitated tricalcium phosphate, and calcium chloride in the production of eggs when fed to hens as supplements to a wheat, yellow corn, and skim-milk ration.

The results indicate that calcium carbonate is the most effective of the materials tried, judging by the degree to which it was utilized by the hens in the production of eggs, its influence on the weight of the egg contents and shells, and the quantity consumed.

Calcium sulphate is not so effective as calcium carbonate, as shown by a smaller egg production and lower weight of egg contents and shells produced.

The hens seem to utilize calcium lactate readily, as shown by the number of eggs produced. However, the quantity of calcium lactate consumed was variable and small when compared to the consumption of calcium carbonate or calcium sulphate.

Calcium chloride was not consumed by the hens in sufficient quantity to justify definite conclusions. Its addition to the basal ration caused only a very small increase in egg production.

Precipitated tricalcium phosphate does not serve as a source of calcium in the production of eggs to the extent that calcium carbonate does, as shown by the number of eggs produced and by their weight.

# ACETIC ACID AS A SOIL DISINFECTANT<sup>1</sup>

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## INTRODUCTION

Acetic acid, in vinegar, is one of the most anciently used preservatives. Its toxicity to bacteria and to fungi has been recognized. Wolf and Shunk (26)<sup>2</sup> found that it was more toxic to the six species of bacteria investigated by them than was hydrochloric, sulphuric, citric, tartaric, malic, or formic acids. In the experiments of Bitting (4) acetic acid was as toxic to fungi as benzoic, boric, or salicylic acids, and it killed *Penicillium expansum*, *Alternaria solani*, and *Oidium lactis* under conditions in which citric, lactic, malic, and tartaric acids only slightly retarded their growth. According to Rideal and Rideal (20), acetic acid 0.3 per cent kills *Bacillus typhosus*. Uppal (24) found that it was toxic to the conidia of *Phytophthora colocasiae*. Wüthrich (27) reported that 0.01 N acetic acid prevented the germination of the conidia of *Phytophthora infestans* and *Plasmopara viticola* and the spores of *Ustilago carbo*. Results secured by Piemeisel (16) led him to believe that it is acetic acid which kills the spores of *Ustilago zeae* in silage. Acetic acid, 0.1 per cent, was found by Hitchcock and Carleton (11) to prevent the germination of urediniospores of *Puccinia coronata*.

Acetic acid may have a toxic or a stimulatory effect on higher plants. In the experiments of Lövinson (14) it retarded the germination of peas. Carr and Haverkamp (6) found that the weights of plants decreased as the quantity of acetic acid, added to the soil in which they were growing, was increased. Heald (10) found that the growth of corn seedlings was prevented by a certain concentration of acetic acid, but that this concentration was greater than the concentration of hydrochloric or sulphuric acids which is toxic to these plants.

In the experiments of Small (21) a much larger percentage of cuttings of rose, privet, and veronica rooted in acetic acid, 0.01 per cent, than in water, and root systems became larger in the acid medium. Promsy (17, 18) found that the germination of seeds was hastened and the growth of seedlings increased by the application of acetic acid to the sand in which they were growing.

The toxicity of acetic acid has been considered or explained by True (23), Kahlenberg and True (13), Heald (10), Winslow and Lochridge (25), Reichel (19), Dunn (8), Cohen and Clark (7), and Uppal (24). Apparently, the toxicity of acetic acid is due partly to the hydrogen ion and largely to the undissociated molecule. Kahlenberg and True (13) found that undissociated acetic acid is toxic, and, according to Heald (10) and True (23), the  $C_2H_3O_2$  ions are not

<sup>1</sup> Received for publication Dec. 5, 1927; issued April, 1928. Published with the approval of the director of the Massachusetts Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 279.

toxic. Wolf and Shunk (26) found that the limit for growth of *Bacterium tabacum* was  $P_H$  5.9 when the medium was adjusted with acetic acid and  $P_H$  4.6 when the medium was adjusted with malic acid. They concluded that the hydrogen ion alone is not responsible for the toxicity of acetic acid.

Soil may be changed chemically as well as biologically by the application of acetic acid. The rate of solubility of soils was found by Bouyoucos (5) to be greater after treatment with acetic acid than after treatment with nitric, hydrochloric, or sulphuric acids. According to Carr and Haverkamp (6), acetic acid forms soluble and toxic salts with iron and aluminum in the soil, and they considered this to be the cause of the toxicity of acetic acid to plants.

There is, then, evidence that acetic acid is fungicidal and bactericidal, and that it may be either toxic or stimulatory to plants. In these effects it is not unlike formaldehyde.

In this paper, which is a preliminary report, results of experiments are presented which show that acetic acid applied to the soil protected plants against diseases caused by fungi in the soil and at a cost about half that of formaldehyde.

#### PREVENTION OF BLACK ROOT ROT OF TOBACCO BY ACETIC ACID

The fungus *Thielavia basicola* (Berk. and Br.) Zopf causes severe black root rot of tobacco in some of the less acid soils in the Connecticut Valley. This disease and bed rot or damping off are combated in the tobacco seed beds by treating the soil with steam or with formaldehyde.

Soil known to be infested with *Thielavia basicola* was placed in 2-gallon crocks, and to it was applied 7, 14, and 21 c. c. normal acetic acid per 100 gm. dry weight of soil, with treatments in duplicate. With the acid, enough water was added to bring the soil to 60 per cent of its water-holding capacity, and at that degree of saturation it was maintained.

Tobacco seeds planted in this soil four weeks after the application of the acid germinated well. While the heaviest application of acetic acid interfered somewhat with growth, the beneficial effect of appropriate quantities was apparent. When the average area of leaves of control plants was 0.37 square inch, the average area of leaves of plants in soil treated with acetic acid (7 c. c. normal acid per 100 gm. soil) was 1.86 square inches (planimeter measurements), or five times as large as that of the controls.

When these plants were 7 weeks old, their roots were examined for infection by *Thielavia*. The results are recorded under the first series in Table 1. Black root rot was severe on control plants, and infection was prevented by acetic acid.

TABLE 1.—Effect of acetic acid on black root rot of tobacco

Number of cubic centimeters of N/1 acetic acid applied per 100 gm. of soil	Infection of roots of tobacco by <i>Thielavia</i>	
	First series	Second series
0 (Control).....	Severe on all.....	Severe on all.*
7.....	None on 83 per cent, trace on 17 per cent.....	None.....
14.....	None.....	Do.
21.....	do.....	Do.

\* An average of 30 lesions per root.

Tobacco plants, from steamed soil, and therefore free from black root rot, were set in this same soil 12 weeks after the application of the acid. As before, the plants grown in the treated soil grew better than the controls with the exception of those receiving the heaviest application of acetic acid, which was so large as to be somewhat toxic to the plants. Black root rot on control plants seriously interfered with their growth.

Eight weeks after these plants were set, their roots were examined for black root rot; the results are recorded under the second series in Table 1. Infection was severe on all control plants, but there was no infection whatever on plants in soil to which acetic acid had been applied.

Equivalent quantities of citric, tartaric, lactic, and malic acids of the same normality were applied to this soil infested with *Thielavia basicola*, but only acetic acid prevented infection.

It has been found by the writer that orthophosphoric acid applied to soil infested with *Thielavia basicola* is very favorable to infection of tobacco by this fungus. Acetic acid together with orthophosphoric acid was therefore applied to such a soil. The soil was kept watered to 60 per cent of its water-holding capacity, and 18 days after the application of the acids tobacco plants from steamed soil were set in it. Six weeks later their roots were examined for black root rot. Infection was severe on control plants and was very mild on plants in soil to which had been applied 9 c. c. normal acetic acid and 8 c. c. normal orthophosphoric acid per 100 gm. of soil. Infection was much reduced, although not entirely prevented, by acetic acid with orthophosphoric acid, and orthophosphoric acid used alone resulted in infection considerably more severe than on control plants.

In order to secure more information about the relative safety or toxicity of acetic acid to tobacco, acetic acid, 56 per cent, was applied to soil in the field at the rate of 3,000 pounds per acre. Two weeks after the application, tobacco plants were set in this soil. No toxicity to plants resulted, and in the absence of black root rot, plants in soil treated with acetic acid had the same average weight at the end of the season as control plants.

In one series of pot experiments, acetic acid was applied to a well-buffered soil (a water-deposited silt or fine sandy loam) and to a poorly buffered soil (an ice-deposited stony loam). Acetic acid was not toxic to tobacco plants (set one week after the application of the acid) in the well-buffered soil, but was toxic to them in the poorly buffered soil.

#### EFFECT OF ACETIC ACID ON SOIL REACTION

It has been pointed out by Stephenson (22) that such organic acids as acetic acid are too rapidly oxidized in the soil to cause an increase in soil acidity. But since it has been found that acetic acid can prevent black root rot of tobacco, and since Anderson, Osmun, and Doran (2) and Morgan and Anderson (15) have shown the relation which exists between the  $P_H$  value of soil and black root rot of tobacco, it is interesting to see whether or not this effect of acetic acid can be correlated with soil reaction.

Acetic acid was applied to soil having an initial  $P_H$  value of 6.1, and the  $P_H$  values of this soil were determined<sup>3</sup> thereafter at frequent intervals. The results of these determinations are shown in Table 2.

TABLE 2.—Effect of acetic acid on the  $P_H$  value of soil

Number of cubic centimeters of N/1 acetic acid applied per 100 gm. of soil	$P_H$ value of soil after—					
	6 days	12 days	15 days	31 days	41 days	63 days
0 (control).....	6.1	6.1	6.1	6.1	6.1	6.1
7.....	4.9	6.1	6.1	6.1	6.1	6.1
14.....	4.6	5.4	5.9	6.1	6.1	6.1
21.....	4.4	4.6	4.9	5.0	6.1	6.1

The application of acetic acid temporarily increased the acidity of the soil, but within a few days or a few weeks, depending on the quantity used, the  $P_H$  value of the soil to which it was applied reverted to its original value. When normal acetic acid, 7 c. c. per 100 gm. of soil, was applied, the  $P_H$  value of the soil was changed from 6.1 to 4.9, but 12 days after the application of the acid the  $P_H$  value of this soil was back to 6.1. In the case of the application of normal acetic acid, 14 c. c. per 100 gm. of soil, the  $P_H$  value of the soil was changed from 6.1 to 4.6, but 31 days after the application of the acid the  $P_H$  value had reverted to 6.1.

When acetic acid, 56 per cent, was applied to soil in the field at the rate of 3,000 pounds per acre, and determinations made 4, 8, and 10 weeks after the application, the  $P_H$  value of the soil was found unchanged.

When acetic acid was applied to a well buffered soil and to a poorly buffered soil, the  $P_H$  value of neither was changed, as determined 3, 6, and 12 weeks after the application of the acid.

Acetic acid was found to surpass either nitric or sulphuric acid in preventing infection of tobacco by *Thielavia basicola*. But the  $P_H$  value of the soil treated with acetic acid was 5.9, which, in the absence of acetic acid, is favorable to this fungus (2), while soil to which the inorganic acids were applied became considerably more acid ( $P_H$  5.6 to 5.4). This is in agreement with the results of Cohen and Clark (7), who found that the growth of certain organisms was inhibited at  $P_H$  5.45 when acetic acid was used to adjust the reaction of the media, but that these same organisms grew at  $P_H$  5.0 when adjustment was made with hydrochloric acid.

Acetic acid has no lasting effect in increasing soil acidity, and its effect on fungi in the soil is not exerted through the factor of soil reaction.

#### EFFECT OF ACETIC ACID ON GERMINATION AND DAMPING OFF OF LETTUCE, CUCUMBER, TOMATO, AND TOBACCO

Acetic acid, in the concentrations named in Table 3, or formaldehyde, 1 : 50, was applied to a soil known to be infested with species of *Pythium* and *Rhizoctonia*. The soil was 4 inches deep, in flats, and the diluted acetic acid or formaldehyde was applied at the rate of 2 quarts per square foot. The soil was stirred 4 days after appli-

<sup>3</sup> The  $P_H$  values of soil were determined colorimetrically with the Stirlen double wedge comparator using the method described by Anderson and Morgan (1).

cation of the chemicals and 10 days after their application seeds were planted. There were planted per flat and for each treatment 70 cucumber seeds, 100 tomato seeds, and 200 lettuce seeds. An equal weight of tobacco seeds was planted in each flat and for each treatment. The soil in all flats was kept at 70 per cent of its water-holding capacity and the flats were in air of high relative humidity.

When it was evident that all seeds had germinated which were going to germinate, the percentage of seeds which failed to germinate in each treatment was determined. The results are recorded in Table 3.

TABLE 3.—*Effect of acetic acid and of formaldehyde on determination and damping off of cucumber, tomato, and lettuce*

Treatment *		Per cent lost								
Pounds of 56 per cent acetic acid in 50 gallons of water	Per cent acetic acid equivalent	By failure to germinate			By damping-off			By failure to germinate and by damping-off.		
		Cucumber	Tomato	Lettuce	Cucumber	Tomato	Lettuce	Cucumber	Tomato	Lettuce
0.00-----	0.0	50	30	36	25	0	17	75	30	53
2.98-----	.4	26	13	7	6	0	4	32	13	11
5.95-----	.8	11	5	7	3	0	1	14	5	8
8.93-----	1.2	2	3	0	0	0	0	2	3	0
Formaldehyde 1 : 50-----		2	0	0	0	0	0	2	0	0

\* Applied to soil at rate of 2 quarts of the solution per square foot.

In control flats, 50 per cent of the cucumber seed, 30 per cent of the tomato seed, and 36 per cent of the lettuce seed failed to germinate; and the seeds which failed to germinate were found to be attacked by soil fungi. In soil to which 1.2 per cent acetic acid (8.93 pounds of 56 per cent acetic acid in 50 gallons of water) was applied, none of the lettuce seed, only 2 per cent of the cucumber seed, and 3 per cent of the tomato seed failed to germinate.

There was considerable loss of seedlings of cucumber, lettuce, and tobacco (but not tomato) by damping off in untreated soil. Species of *Pythium* and *Rhizoctonia* were present in the damped off seedlings. In the untreated soil 25 per cent of the cucumber seedlings and 17 per cent of the lettuce seedlings damped off. There was no damping off of cucumber and lettuce seedlings in soil to which acetic acid, 1.2 per cent, was applied. The application of this strength of acetic acid was as efficient as formaldehyde in preventing damping off. In the untreated soil 75 per cent of the cucumbers, 30 per cent of the tomatoes, and 53 per cent of the lettuce plants were lost by failure to germinate and by damping off combined. In soil to which 1.2 per cent acetic acid was applied, the loss from these causes was only 2 per cent of the cucumbers, 3 per cent of the tomatoes, and none of the lettuce. Acetic acid 0.8 per cent was nearly as effective in preventing damping off.

In Figure 1 the effect of acetic acid in preventing damping off of tobacco seedlings is evident. Acetic acid 0.4 per cent was insufficient for protection but acetic acid 0.8 or 1.2 per cent prevented damping off.

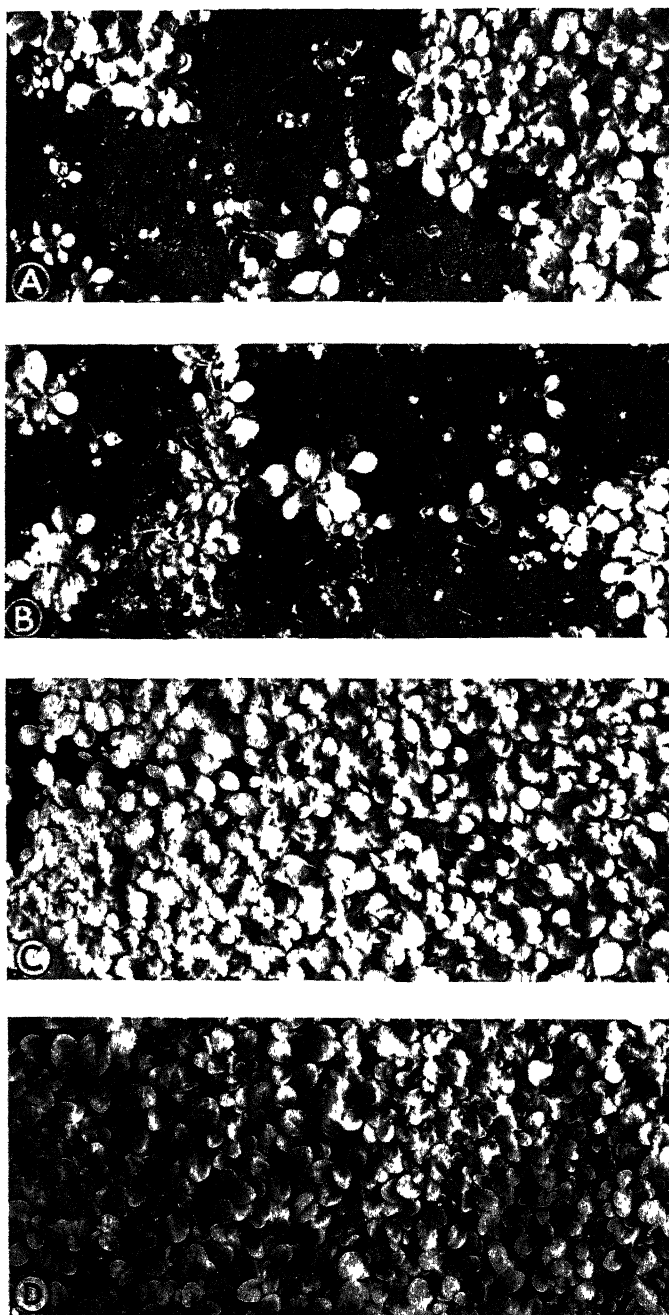


FIG. 1.—Control of damping off of tobacco by the use of acetic acid. A, No acetic acid; B, acetic acid, 0.4 per cent; C, acetic acid, 0.8 per cent; D, acetic acid, 1.2 per cent



Tobacco plants which did not damp off in each of the several treatments were counted. For each 100 plants living in the untreated soil, there were 367 plants living in the soil treated with acetic acid 0.8 per cent and slightly more than this in the soil treated with acetic acid 1.2 per cent, which treatment gave results equal to those secured with formaldehyde.

#### EFFECT OF ACETIC ACID ON BED ROT AND ON BLACK ROOT ROT OF TOBACCO IN SEED BEDS

Acetic acid, at the rate of 2 quarts per square foot of a solution of 7.5 pounds of 56 per cent acetic acid in 50 gallons of water (equivalent to acetic acid 1.008 per cent), was applied to tobacco seed beds known to be infested with *Thielavia basicola* and with bed-rot fungi, *Rhizoctonia solani* and *Pythium* sp.<sup>4</sup>

TABLE 4.—Effect of acetic acid on black root rot and on bed rot of tobacco in seed beds

Seed bed No.	Percent of plants showing black root rot		Number of centers of infection of bed rot	
	In control	In acetic-acid treated area	In control	In acetic-acid treated area
1.....	100	19	0	0
2.....	26	0	15	0
3.....	18	0	0	0

In seed beds No. 1 and No. 2 (see Table 4) tobacco seeds were sowed one week after the application of the acid, and in these plots germination was not as good as in the control plots. In bed No. 3, seed was sowed 20 days after the application of the acid, and germination was better in this plot than in its control plot. On the basis of this and other experiments it seems unsafe to sow tobacco seeds as soon as one week after the application of acetic acid to soil. Many weeds came up in the control plots in these seed beds, but very few weeds came up in the plots to which acetic acid was applied.

Bed rot, caused by *Rhizoctonia solani* and *Pythium* sp., developed in only one seed bed (No. 2). In the control plot of this seed bed, 15 centers of infection appeared and from these bed rot spread rapidly within the plot. There was no bed rot in the plot to which acetic acid was applied. In this and the other seed beds, control plots were of the same area as treated plots and adjacent to them.

The percentages of plants found to be infected with black root rot are recorded in Table 4. In the control plots of seed beds No. 2 and No. 3, 26 and 18 per cent of the plants, respectively, were infected by *Thielavia basicola*. There was no infection in the plots to which acetic acid was applied in these beds. In seed bed No. 1, infection was unusually severe in the control plot, for in it 100 per cent of the plants had black root rot. When acetic acid was applied in seed bed No. 1, there was already so much water in the soil that much—probably one-third—of the solution of acetic acid ran off the bed. Under these conditions black root rot was not entirely prevented by

<sup>4</sup> Acknowledgment is made of the cooperation of Walter W. Sanderson and T. L. Warner, tobacco growers, on whose farm these experiments were conducted.

acetic acid but was reduced to 19 per cent as compared with 100 per cent in the control plot.

In order to learn whether or not dilute acetic acid can be applied to living tobacco plants in the seed bed to control bed rot, acetic acid in several concentrations was sprayed on growing tobacco seedlings among which bed rot had appeared. The spread of the disease was checked, but living plants were injured by the application of a 0.5 per cent solution of the acid. Concentrations so low as not to injure the plants did not prevent the spread of bed rot. The usefulness of acetic acid, as of formaldehyde, for the control of bed rot of tobacco in seed beds is confined to its application to the soil before plants are growing in it.

#### EFFECT OF ACETIC ACID ON DAMPING OFF OF SEEDLINGS OF WHITE SPRUCE

Acetic acid of two concentrations, 8.33 pounds and 12.5 pounds, of 56 per cent acetic acid in 50 gallons of water (equivalent respectively to 1.12 and 1.68 per cent acetic acid) was applied at the rate of 1.64 quarts per square foot to seed beds in a forest nursery.<sup>5</sup> A control seed bed, not treated, was adjacent to them. Seed beds were 44 square feet in area. One-half pound of seeds of white spruce was planted in each bed seven days after treatment.

Although the interval of time between the application of acetic acid and the planting of seeds was relatively short, germination was not injured.\* Germination of white spruce seeds was much better and there were fewer weeds in the beds to which acetic acid was applied than in the control bed. Loss by damping off was severe in the control bed, as may be seen by reference to Figure 2, A. There was very little damping off in the beds to which acetic acid was applied. Four months after seeds were planted, the number of seedlings in 5 representative square feet in each bed were counted. The average number of seedlings per square foot in each bed and the number of seedlings in each seed bed figured from this are recorded in Table 5.

TABLE 5.—*Effect of acetic acid on damping off of white spruce seedlings*

Treatment *		Average number of seedlings		Seedlings per unit area, expressed in relative numbers
Pounds of 56 per cent acetic acid in 50 gallons of water	Equivalent per cent of acetic acid	Per square foot	Per seed bed	
8.33.....	1.12	746	32,824	315
12.50.....	1.68	665	29,260	281
0 (control).....	0	237	10,428	100

\* 1.64 quarts of diluted acetic acid per square foot.

For each 100 seedlings in the untreated soil there were 315 seedlings in soil to which 1.12 per cent acetic acid was applied. This concentration of acetic acid gave better results than the higher concentration (1.68 per cent acetic acid) for in soil to which the higher con-

<sup>5</sup> The cooperation of John Palmer, superintendent of the State forest nursery in Amherst, Mass., is acknowledged.

centration was applied there were 281 seedlings for each 100 seedlings in untreated soil.

The stands in the several seed beds three months after seeds were sowed may be compared by reference to Figure 2. The control of damping off secured by both concentrations of acetic acid is evident, and it is likewise evident that the optimum concentration of acetic

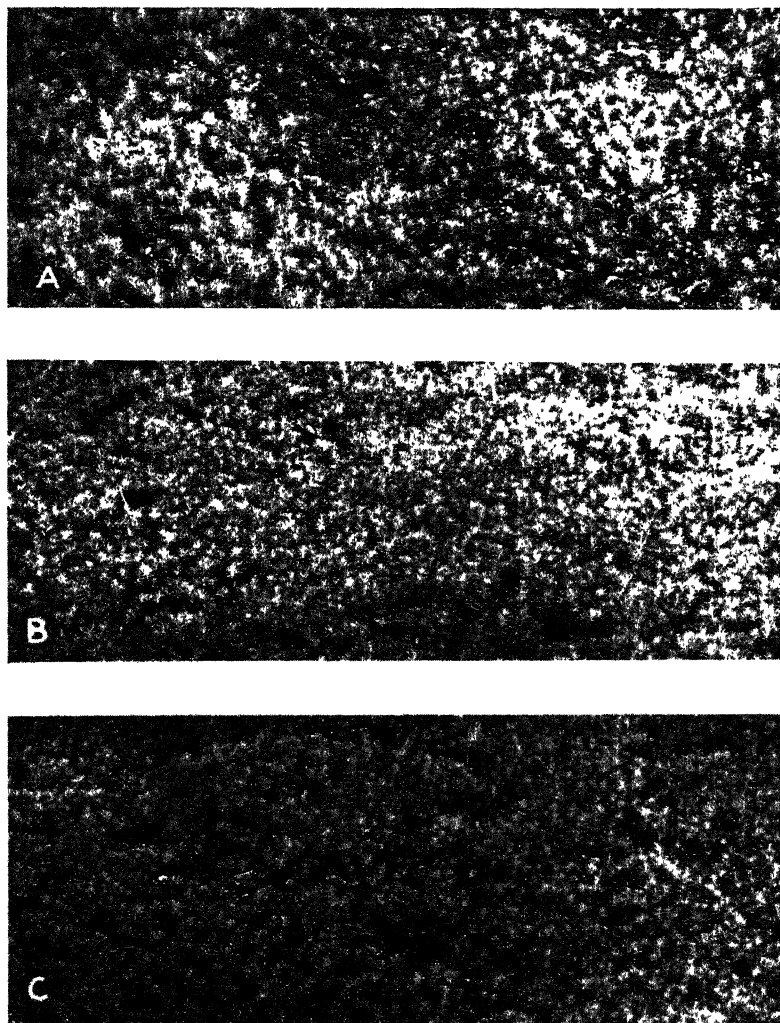


FIG. 2.—Control of damping off of white spruce seedlings by the use of acetic acid. A, No acetic acid; B, acetic acid, 1.12 per cent; C, acetic acid, 1.68 per cent

acid is nearer 1.12 per cent than 1.68 per cent. The fact that acetic acid 1.12 per cent used in this way protected seedlings of white spruce against damping off without injuring the seeds or seedlings is of interest because of the results secured by Hansen, Kenety, Wiggin, and Stakman (9). In their experiments, the application of sulphuric acid, hydrochloric acid, or formaldehyde greatly reduced the ger-

mination of seeds of white spruce, and they considered as questionable the use of any of the common fungicides for the prevention of damping off of seedlings of this species.

#### EFFECT OF ACETIC ACID ON BROWN ROOT ROT OF TOBACCO

When acetic acid was applied to "brown root-rot soil," that is, soil known to have in it the cause of brown root rot, tobacco plants subsequently set in the treated soil grew without showing symptoms of brown root rot. Johnson, Slagg, and Murwin (12) found that when formaldehyde was similarly applied to brown root-rot soil, the ability of this soil to produce symptoms of brown root rot of tobacco was destroyed.

The cause of brown root rot is at present unknown, and the results with acetic acid are presented here not because of any light they may shed on the nature of the disease, but because there is in them additional evidence of the effectiveness of acetic acid as a substitute for formaldehyde for soil treatment.

Tobacco seeds were sowed in pots of brown root-rot soil three weeks after the application to it of 1 per cent acetic acid. Seeds were also sowed in pots of this soil without acetic acid. Brown root rot was found on seedlings in untreated soil when they were 4 weeks old. Roots of all plants were examined when they were 9 weeks old. Brown root rot was found to be severe on all plants in untreated soil, but there was not even a trace of the disease on plants in soil to which acetic acid had been applied.

The severity of brown root rot on plants in untreated soil seriously retarded their growth. When the average dry weight of plants in untreated soil was 0.90 gm., the average dry weight of plants in soil treated with acetic acid was 4.76 gm., or more than five times as great as that of the controls.

#### COST OF USING ACETIC ACID COMPARED WITH COST OF USING FORMALDEHYDE

A cheaper chemical than formaldehyde for the partial sterilization of soil is desirable. Beach (3) applied formaldehyde at the usual rate and reported that it cost (in 1926) \$0.21 for the 1.4 pounds used to treat 18 square feet of soil, or \$508 per acre.

One-half gallon of dilute acetic acid per square foot of soil has been found to be sufficient, and this is the rate at which formaldehyde 1 : 50 is usually applied. The relative cost of these chemicals may therefore be compared by comparing the cost of 50 gallons of each.

Formaldehyde 1 : 50 contains 1 gallon (9.1 pounds) of the commercial 40 per cent solution of formaldehyde in 50 gallons of water. This is now purchasable (in lots of 100 pounds) for about \$0.12 per pound. The cost of formaldehyde for 50 gallons (enough for 100 square feet) is therefore \$1.09 and the cost of formaldehyde for 1 acre is \$475.

The concentrations of acetic acid which were found to control the plant diseases named in this paper were 1.2 to 1 per cent. These are equivalent to 8.93 to 7.44 pounds of 56 per cent acetic acid in 50 gallons of water. Acetic acid of several different concentrations (28 to 99 per cent) is sold, but the cost per unit of actual acetic acid is not greatly different. Acetic acid 56 per cent is now sold (in lots

of 100 pounds) for \$0.066 per pound. When 8 pounds of this chemical is used in 50 gallons of water (equivalent to 1.07 per cent acetic acid) the cost of acetic acid for 50 gallons (enough for 100 square feet) is \$0.53 and the cost of acetic acid for 1 acre is \$231.

The cost of acetic acid is therefore about 49 per cent of the cost of formaldehyde for soil treatment.

#### SUMMARY

The application to soil of 1 to 1.2 per cent acetic acid (equivalent to 7.44 to 8.93 pounds of 56 per cent acetic acid in 50 gallons of water) at the rate of about one-half gallon per square foot was found to protect tobacco against black root rot, brown root rot, and bed rot or damping off; and to protect cucumber, tomato, lettuce, and white spruce against injury by damping off during and after seed germination.

For the treatment of a unit area of soil, the cost of acetic acid is about 49 per cent of the cost of formaldehyde.

Acetic acid is toxic to seeds and plants with which it comes in contact. The exact time interval which must elapse between the application of the chemical to soil and the planting of seed will depend upon the species of plant and upon the soil; 14, 10, or, in some cases, 7 days have been long enough.

Acetic acid has no lasting effect on the reaction, i. e., the  $P_H$  value of soil to which it is applied.

#### LITERATURE CITED

- (1) ANDERSON, P. J., and MORGAN, M. F.  
1926. BLACK ROOTROT AND SOIL REACTION. Conn. Agr. Expt. Sta., Tobacco Sta. Bul. 6: 59T-66T, illus.
- (2) ——— OSMUN, A. V., and DORAN, W. L.  
1926. SOIL REACTION AND BLACK ROOTROT OF TOBACCO. Mass. Agr. Expt. Sta. Bul. 229, p. [117]-[136], illus.
- (3) BEACH, W. S.  
1926. FORMALDEHYDE AND OTHER DISINFECTANTS IN THE CONTROL OF LETTUCE DISEASES. Penn. Agr. Expt. Sta. Bul. 202, 28 p., illus.
- (4) BITTING, K. G.  
1920. THE EFFECT OF CERTAIN AGENTS ON THE DEVELOPMENT OF SOME MOULDS. 176 p., illus., Washington, D. C.
- (5) BOUYOUCOS, G. J.  
1919. RATE AND EXTENT OF SOLUBILITY OF SOILS UNDER DIFFERENT TREATMENTS AND CONDITIONS. Mich. Agr. Expt. Sta. Tech. Bul. 44, p. [681]-727, illus.
- (6) CARR, R. H., and HAVERCAMP, H. G.  
1924. MODIFICATIONS OF PLANT GROWTH AND ASH CONTENT AS EFFECTED BY ACIDS ADDED TO SOILS. Jour. Amer. Soc. Agron. 16: 278-283.
- (7) COHEN, B., and CLARK, W. M.  
1919. THE GROWTH OF CERTAIN BACTERIA IN MEDIA OF DIFFERENT HYDROGEN ION CONCENTRATIONS. Jour. Bact. 4: 409-427, illus.
- (8) DUNN, M. S.  
1926. EFFECTS OF CERTAIN ACIDS AND THEIR SODIUM SALTS UPON THE GROWTH OF *SCLEROTINIA CINEREA*. Amer. Jour. Bot. 13: 40-58, illus.
- (9) HANSEN, T. S., KENETY, W. H., WIGGIN, G. H., and STAKMAN, E. C.  
1923. A STUDY OF THE DAMPING-OFF DISEASE OF CONIFEROUS SEEDLINGS. Minn. Agr. Expt. Sta. Tech. Bul. 15, 35 p., illus.
- (10) HEALD, F. D.  
1896. ON THE TOXIC EFFECT OF DILUTE SOLUTIONS OF ACIDS AND SALTS UPON PLANTS. Bot. Gaz. 22: 125-153, illus.

- (11) HITCHCOCK, A. S., and CARLETON, M. A.  
1893. PRELIMINARY REPORT ON RUSTS OF GRAIN. *Kans. Agr. Expt. Sta. Bul.* 38, 14 p., illus.
- (12) JOHNSON, J., SLAGG, C. M., and MURWIN, H. F.  
1926. THE BROWN ROOTROT OF TOBACCO AND OTHER PLANTS. *U. S. Dept. Agr. Bul.* 1410, 30 p., illus.
- (13) KAHLBERG, L., and TRUE, R. H.  
1896. ON THE TOXIC ACTION OF DISSOLVED SALTS AND THEIR ELECTROLYTIC DISSOCIATION. *Bot. Gaz.* 22: 81-124.
- (14) LÖVINSON, O.  
1900. UEBER KEIMUNGS- UND WACHSTHUMSVERSUCHE AN ERBSEN IN LÖSUNGEN VON FETTSÄUREN SALZEN UNTER AUSSCHLUSS VON MINERALSÄUREN. *Bot. Centbl.* 83:1-12, 33-43, 65-75, 97-106, 129-138, 185-195, [209]-224, illus.
- (15) MORGAN, M. F., and ANDERSON, P. J.  
1927. RELATION OF SOIL REACTION TO BLACK ROOTROT AND GOOD TOBACCO. *Conn. Agr. Expt. Sta., Tobacco Sta. Bul.* 8: 47T-49T, illus.
- (16) PIEMEISEL, F. J.  
1917. FACTORS AFFECTING THE PARASITISM OF *USTILAGO ZEÆ*. *Phytopathology* 7: [294]-307.
- (17) PROMSKY, G.  
1911. DE L'INFLUENCE DE L'ACIDITÉ SUR LA GERMINATION. *Comp. Rend. Acad. Sci. [Paris]* 152: 450-452.
- (18) ———  
1912. RÔLE DES ACIDES DANS LA GERMINATION. *Bul. Soc. Natl. Agr. France* 72: 916-922.
- (19) REICHEL, J.  
1910. ÜBER DAS VERHALTEN VON *PENICILLIUM* GEGENÜBER DER ESSIGSÄURE UND IHREN SALZEN. *Biochem. Ztschr.* 30: [152]-159.
- (20) RIDEAL, S., and RIDEAL, E. K.  
1921. CHEMICAL DISINFECTION AND STERILIZATION. 313 p., illus. London.
- (21) SMALL, J.  
1923. PROPAGATION BY CUTTINGS IN ACIDIC MEDIA. *Gard. Chron.* (3) 73: 244-245, illus.
- (22) STEPHENSON, R. E.  
1921. THE EFFECT OF ORGANIC MATTER ON SOIL REACTION, II. *Soil Sci.* 12: 145-162, illus.
- (23) TRUE, R. H.  
1900. THE TOXIC ACTION OF A SERIES OF ACIDS AND OF THEIR SODIUM SALTS ON *LUPINUS ALBUS*. *Amer. Jour. Sci.* (4) 9: 183-192.
- (24) UPPAL, B. N.  
1926. TOXICITY OF ORGANIC COMPOUNDS TO THE SPORES OF *PHYTOPHTHORA COLOCASIAE* RAC. *Jour. Agr. Research* 32: 1069-1097.
- (25) WINSLOW, C.-E. A., and LOCHRIDGE, E. E.  
1906. THE TOXIC EFFECT OF CERTAIN ACIDS UPON TYPHOID AND COLON BACILLI IN RELATION TO THE DEGREE OF THEIR DISSOCIATION. *Jour. Infect. Diseases* 3: 547-571.
- (26) WOLF, F. A., and SHUNK, I. V.  
1921. TOLERANCE TO ACIDS OF CERTAIN BACTERIAL PLANT PATHOGENES. *Phytopathology* 11: [244]-250.
- (27) WÜTHRICH, E.  
1892. UEBER DIE EINWIRKUNG VON METALLSALZEN UND SÄUREN AUF DIE KEIMFÄHIGKEIT DER SPOREN EINIGER DER VERBREITETSTEN PARASITISCHEN PILZE UNSERER KULTURPFLANZEN. *Ztschr. Pflanzenkrank.* 2: 16-31.

# THE GROWTH OF TOBACCO AND BROWN ROOT ROT OF TOBACCO AS AFFECTED BY TIMOTHY INFUSIONS OF DIFFERENT AGES<sup>1</sup>

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## INTRODUCTION

When tobacco is grown after timothy or certain other plants, used either as a cover crop or in the rotation, the growth of the tobacco is often retarded, and a certain accompanying type of injury to the roots of the tobacco has given rise to the name "brown root rot." This apparent injurious effect on tobacco of the decomposing residue of timothy in the soil has frequently been observed and recorded in the literature (1, 2, 7, 11, 12).<sup>2</sup>

But this injurious effect of timothy on the tobacco which follows it has been found to be usually transitory by those who have investigated it in field experiments (1, 2, 7, 11); that is, the injurious factor which is associated with timothy soon becomes inactive. When the use of a timothy cover crop was discontinued in the experiments of Anderson, Osmun, and Doran (2), its previously harmful effect on tobacco quickly disappeared and the yield of tobacco on those plots which had had a timothy cover crop became greater by 17 per cent than on those which had not. Fall plowing of grass sod is reported by Anderson (1) to have reduced the injurious effect of sod on tobacco, and in his experiments there was, on plots not fertilized, a difference in favor of plowing under cover crops very early in the spring instead of six weeks later.

The direct cause of brown root rot is at present not known. In the investigations of Johnson, Slagg, and Murwin (11), no causal organism was demonstrated to be associated with it.

One hypothesis is that brown root rot is the expression of the toxic effect on roots of tobacco of the decomposition products of certain preceding crops. If such is the case it is to be expected that the effect on tobacco plants of the residues of these preceding crops will depend on the stage of their decomposition. As Martin (13) has pointed out, the breakdown of vegetable organic matter includes several distinct steps and consequent intermediary products, and Gardner (6) concluded that toxins which result from and during the decomposition of plant residues are, in subsequent stages of the decomposition process, rendered harmless.

Experiments here described were undertaken with the object of learning whether or not there is a relation, such as field experiments have indicated may exist, between brown root rot of tobacco and the stage of decomposition of timothy.

This paper is a record of progress and the results reported should not be considered conclusive.

<sup>1</sup> Received for publication Jan. 9, 1923; issued April, 1923. Published with the approval of the director, Massachusetts Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 287.

## METHOD

Infusions of timothy plants were made at intervals of one week. Infusions differed only in age and each consisted of the timothy from 2 square feet of sod, washed free of soil and placed in 10 liters of tap water in open jars. Entire timothy plants were used in the first experiment. In the second experiment the tops of the timothy which was used had been kept clipped during its growth. In the first experiment the infusions were stored at about 27° C. and in the second experiment they were stored at about 16° C. To insure the absence of brown root rot, except such as might be due to the application of the infusions, soil was steamed (11). Seven kilograms of soil was placed in each of the 2-gallon pots used and five young tobacco seedlings from steamed soil were set in each pot. Treatments were in duplicate so that for each treatment there were 10 plants. No fertilizer was used. The soil in all pots was kept at 60 per cent of its water-holding capacity.

To the soil in each pot, 1 liter of timothy infusion of the ages shown in Tables 1 and 2 was applied when plants were set and 250 c. c. of an infusion of the same age was applied to each pot each week thereafter. Pot No. 1 always received an infusion 1 week old, pot No. 2 an infusion 2 weeks old, and so on. A portion of the infusion which was 1 week old at the first application was applied the next week to other plants as an infusion 2 weeks old, and so on, through the series. All the pots of plants therefore received some of the infusion, but in each case the infusion was of a different age.

## EFFECT OF TIMOTHY INFUSIONS ON TOBACCO IN THE FIRST EXPERIMENT

As early as three weeks after plants were set, it was apparent that those in soil receiving infusions 5 to 8 weeks old, inclusive, were making a much poorer growth than plants which were receiving timothy infusions 1, 2, or 3 weeks old.

The writer has several times observed that the symptoms of brown root rot on tobacco seedlings even in "brown root-rot soil" may appear as a retardation of growth several days or even two weeks before the brown discolorations and lesions appear on the roots. This in itself may have some significance as an indication of the nature of the cause of brown root rot when considered in connection with certain other evidence presented in this paper.

Because of the delay which there may be in the appearance of the symptoms on the roots, plants were not removed from the soil until they had been receiving applications of infusions for nine weeks. At that time, they were washed, the roots examined, and the plants dried to constant weight and weighed. The average dry weight per plant in each treatment is recorded in Table 1.



TABLE 1.—*Effect of timothy infusions of different ages on dry weights of tobacco plants in the first experiment*

Age in weeks of timothy infusion applied	Average dry weight (grams) per plant <sup>a</sup>		Dry weights expressed in relative numbers	
	In each treatment	Treatments grouped	In each treatment	Treatments grouped
0 <sup>b</sup> .....	4.23	4.23	100	100
1.....	3.96	4.41	94	104
2.....	4.53		107	
3.....	4.76		112	
4.....	3.08	3.08	73	73
5.....	.45	.91	11	20
6.....	1.38		30	
7.....	.08	.08	2	2
8.....	1.24	1.24	29	29
9.....	2.58	2.92	61	69
10.....	3.27		77	

<sup>a</sup> Average of 10 plants per treatment.<sup>b</sup> Water only.

The outstanding result was that the effect of infusions of timothy on the growth of tobacco was found to depend entirely on the age of the infusion. In the comparisons shown in Table 1 dry weights are expressed as relative numbers. Plants treated with infusions 1 to 3 weeks old, inclusive, had an average relative weight of 104 as compared with 100 for plants which received only water. It is therefore apparent that infusions 1 to 3 weeks old did not interfere with the growth of tobacco. Plants to which infusions 4 weeks old were applied had an average relative weight of 73. The application of infusions 5 and 6 weeks old resulted in much smaller plants, with an average relative weight of 20. The smallest plants, which had an average relative weight of only 2, were those to which timothy infusions 7 weeks old were applied. The infusion 8 weeks old was less injurious and plants to which it was applied had an average relative weight of 29. Plants which received infusions 9 and 10 weeks old were significantly larger, with an average relative weight of 69.

Roots on plants which received only water or timothy infusions 1, 2, and 3 weeks old were white and apparently normal.

The roots of all plants in the other treatments were more or less discolored and showed distinct or coalescing brown lesions. In the absence of a proven parasite, the identification of brown root rot depends on symptoms, macroscopic or microscopic. The injury to roots of tobacco plants to which infusions of timothy of certain ages were applied did not differ from the symptoms of brown root rot as it has been described (11) and as the writer recognizes it in the field.

There was only a trace of such injury on roots of plants which received infusions 4 weeks old. It was most severe on roots of plants which received infusions 5 to 8 weeks old, inclusive, and was relatively mild on roots of plants which received infusions 9 and 10 weeks old. As in the case of growth, root injury or freedom from it depended on the age of the timothy infusions applied, and the trends of growth depression and root injury were practically parallel.

Other investigators have observed the discoloration or browning of roots of other plants which resulted from similar treatments. Roots

of plants to which Collison (4) applied water extracts of straw usually became brown, and Hill (9) observed that the roots of plants which received products of the decomposition of cellulose became discolored.

# EFFECT OF TIMOTHY INFUSIONS ON TOBACCO IN THE SECOND EXPERIMENT

When the plants in the second experiment had been growing 4 weeks it was apparent that plants which were receiving infusions 2 to 6 weeks old, inclusive, were making a very much better growth and that those plants which were receiving infusions 9 to 12 weeks old, inclusive, were making a poorer growth than plants receiving only water. Two plants were removed from each pot at this time. There were some brown lesions on the roots of the plants the growth of which was retarded, but the injury was not as pronounced as when the remaining plants were examined four weeks later.

The average dry weights of the tobacco plants after timothy infusions had been applied to them eight weeks are recorded in Table 2.

TABLE 2.—*Effect of timothy infusions of different ages on dry weights of tobacco plants in the second experiment*

Age in weeks of timothy infusion applied	Average dry weight (grams) per plant <sup>a</sup>		Dry weights expressed in relative numbers	
	In each treatment	Treatments grouped	In each treatment	Treatments grouped
0 <sup>b</sup> .....	0.11	0.11	100	100
1.....	.05	.05	45	45
2.....	3.92	4.46	3,563	4,054
3.....	3.46		3,145	
4.....	5.57		5,063	
5.....	5.06		4,600	
6.....	4.29		3,900	
7.....	.66	.66	600	600
8.....	.42	.42	381	381
9.....	.09	.09	82	82
10.....	.05	.05	45	48
11.....	.05		45	
12.....	.06		54	

<sup>a</sup> Average of 6 plants in each treatment.

<sup>b</sup> Water only.

The plants did not make a good growth in pots to which only water was applied. The soil was rather poor and no fertilizer was used. But, as may be seen by reference to Table 2, the nature of the effect of the timothy infusions on the growth of tobacco depended, as in the first experiment, on the age of the infusions and hence the stage of the decomposition of the timothy.

However, reference to Tables 1 and 2 will show that a timothy infusion of a certain age did not have the same effect on plants in both experiments. This is not surprising, for the infusions were prepared differently. The proportion of roots to tops of timothy used was greater in the second than in the first experiment, and Garner, Lunn, and Brown (7) have found that the roots and tops of preceding plants do not have the same effect on tobacco. Furthermore, the infusions used in the first experiment were stored at a temperature higher by 11° C. than that in the second experiment,

and it is well known that bacterial action similar to that of which such a decomposition is the result, is much affected by temperature.

As shown in Table 2, comparisons of dry weights are expressed as relative numbers, 100 being taken as the average relative weight of plants which received only water. On this basis the average relative weight of plants to which a timothy infusion 1 week old was applied was 45. Plants were greatly benefited by the application of infusions 2 to 6 weeks old, inclusive, so that their average relative weight was 4,054. There was a slight but greatly diminished beneficial effect in the case of plants which received timothy infusions 7 and 8 weeks old, for their average relative weights were 600 and 381, respectively. Infusions 8 weeks old retarded growth considerably and resulted in an average relative weight of 82. Infusions 10 to 12 weeks old, inclusive, were very injurious and the plants to which they were applied had an average relative weight of only 48.

Roots of plants to which only water was applied were white although small. Roots of plants which received timothy infusion 2 to 6 weeks old, inclusive, were white and showed no trace of injury. There were brown lesions and injury, apparently the same as in the first experiment, on roots of plants which received infusions 1 week and 7 to 12 weeks old, inclusive. This injury was most severe on roots of plants which received infusions 1, 9, 10, 11, and 12 weeks old.

#### DISCUSSION

When infusions of timothy were prepared as in the first experiment, the products of the decomposition of timothy during the first three weeks of the decomposition process were harmless or slightly beneficial to tobacco. These decomposition products were increasingly injurious to tobacco from the fourth to the seventh week, and decreasingly injurious from the seventh to the tenth week of the decomposition. When infusions were prepared by the method used in the second experiment, the products of the decomposition of timothy were injurious the first week, very beneficial from the third to the sixth week, decreasingly beneficial in the seventh and eighth weeks, and decidedly injurious to tobacco in the tenth, eleventh, and twelfth week of the decomposition process. In both experiments the products of the decomposition of timothy were at times innocuous and at times harmful to tobacco, depending on the age of the infusions.

The relation of the response of plants to the stage of the decomposition of vegetable organic matter applied to them has been noted by other investigators. Breazeale (3) found that the nature of the effect on plants of the decomposition products of sorghum depends on how far the decomposition process has progressed. In his experiments Collison (4) found that the effect produced by extracts of straw on seedlings when fresh extracts were used was different from the effect produced when he used old extracts. Hartwell and Pember (8) found that in the early stages of the decomposition of oats, the effect on other plants was unfavorable, but that in later stages of the decomposition there was a favorable effect. Gardner (6) concluded that some organic compounds which when fresh and unchanged may be actually toxic to plants are, in most soils, eventually decomposed or otherwise rendered nontoxic, usually by organisms.

But Gardner concedes that some toxins may be decomposed by chemical agencies and this point also is of interest, for formalin has been found (11) to destroy the ability of "brown root-rot soil" to produce the disease, and the writer has found that soil treatment with acetic acid has a similar effect.

The fact that only at certain stages in its decomposition did timothy retard the growth of tobacco and apparently induce brown root rot seems to the writer to lend support to the hypothesis that brown root rot may be only the expression on tobacco roots of the injurious effect of the preceding crop. It has been found (11) that merely drying or aerating "brown root-rot soil" frees it of the cause of the disease, and this, too, is in agreement with the hypothesis that brown root rot is caused by some toxic substance which is easily volatilized, decomposed, or otherwise rendered innocuous. Several previous investigators (4, 5, 9) have found that decomposing plant residues contain substances injurious to the roots of plants, and Collison (4) concluded that the residues of timothy and certain other plants may be important contributors to soils of compounds toxic to plant growth.

Timothy was used in these experiments because it is one of the most injurious crops in its effect on tobacco in the field. Collison (4) observed that extracts of timothy were more injurious to the plants used by him than were the extracts of wheat or oats. There is a possibility that this may be explained by the relative slowness with which timothy decomposes and the consequently long persistence of toxic substances associated with its decomposition. Nonlegumes in general have been found (9) to decompose more slowly than legumes, and the decomposition of timothy residues has been found (14) to extend over a longer period than that of clover residues.

In the experiments here described infusions of certain ages were found to be beneficial rather than harmful to tobacco. It was one of the conclusions of Jensen (10) and of Martin (13) that some of the products of the decay of plant substance may render available to plants certain otherwise insoluble nutrients in the soil. The response of tobacco plants in these experiments showed that at some stage or stages in the decomposition of timothy there are products formed which are favorable to tobacco and which certainly do not induce brown root rot.

#### SUMMARY

When infusions of timothy were applied to soil in which tobacco plants were growing, the effect on the tobacco was found to be sometimes harmless and sometimes very injurious, depending on the age of the infusions; that is, on the stage of the decomposition of the timothy.

The response of plants to an infusion of a given age was found to be influenced by the proportion of tops to roots of timothy used or by the temperature at which the decomposition process went on.

Those infusions of timothy which retarded the growth of tobacco plants produced on their roots brown discolorations and lesions apparently of the same type as those which, in the field, have given rise to the name "brown root rot."

These results lend support to the hypothesis that brown root rot of tobacco is the expression of the injurious effect on tobacco roots of one or more toxic substances which are formed from, and at certain stages in, the decomposition of vegetable organic matter, more especially the residues of certain slowly decomposing crops such as timothy.

#### LITERATURE CITED

- (1) ANDERSON, P. J.  
1926. COVER CROPS FOR TOBACCO. Conn. Agr. Expt. Sta., Tobacco Sta. Bul. 6: 55T-59T.
- (2) ——— OSMUN, A. V., and DORAN, W. L.  
1926. SOIL REACTION AND BLACK ROOT ROT OF TOBACCO. Mass. Agr. Expt. Sta. Bul. 229, p. [117]-[136], illus.
- (3) BREAZEALE, J. F.  
1924. THE INJURIOUS AFTER-EFFECTS OF SORGHUM. Jour. Amer. Soc. Agron. 16: 689-700, illus.
- (4) COLLISON, R. C.  
1925. THE PRESENCE OF CERTAIN ORGANIC COMPOUNDS IN PLANTS AND THEIR RELATION TO THE GROWTH OF OTHER PLANTS. Jour. Amer. Soc. Agron. 17: 58-68.
- (5) ——— and CONN, H. J.  
1925. THE EFFECT OF STRAW ON PLANT GROWTH. N. Y. State Agr. Expt. Sta. Tech. Bul. 114, 35 p., illus.
- (6) GARDNER, W. A.  
1926. THE DECOMPOSITION OF TOXINS BY SOIL ORGANISMS. Ala. Agr. Expt. Sta. Bul. 225, 38 p.
- (7) GARNER, W. W., LUNN, W. M., and BROWN, D. E.  
1925. EFFECTS OF CROPS ON THE YIELDS OF SUCCEEDING CROPS IN THE ROTATION, WITH SPECIAL REFERENCE TO TOBACCO. Jour. Agr. Research 30: 1095-1132, illus.
- (8) HARTWELL, B. L., and PEMBER, F. R.  
1924. THE EFFECT IN POT CULTURE OF GREEN MANURE IN DIFFERENT STAGES OF GROWTH AND DECOMPOSITION, ON THE SUBSEQUENT CROP. Jour. Amer. Soc. Agron. 16: 750-753.
- (9) HILL, H. H.  
1926. DECOMPOSITION OF ORGANIC MATTER IN SOIL. Jour. Agr. Research 33: 77-99, illus.
- (10) JENSEN, C. A.  
1917. EFFECT OF DECOMPOSING ORGANIC MATTER ON THE SOLUBILITY OF CERTAIN INORGANIC CONSTITUENTS OF THE SOIL. Jour. Agr. Research 9: 253-268.
- (11) JOHNSON, J., SLAGG, C. M., and MURWIN, H. F.  
1926. THE BROWN ROOT ROT OF TOBACCO AND OTHER PLANTS. U. S. Dept. Agr. Bul. 1410, 30 p., illus.
- (12) JONES, J. P.  
1927. REPORT OF PROGRESS IN TOBACCO INVESTIGATION. Mass. Agr. Expt. Sta. Circ. 74, 8 p.
- (13) MARTIN, T. L.  
1921. DECOMPOSITION OF GREEN MANURES AT DIFFERENT STAGES OF GROWTH. N. Y. Cornell Agr. Expt. Sta. Bul. 406, p. 139-169, illus.
- (14) WILSON, B. D., and WILSON, J. K.  
1925. AN EXPLANATION FOR THE RELATIVE EFFECTS OF TIMOTHY AND CLOVER RESIDUES IN THE SOIL ON NITRATE DEPRESSION. N. Y. Cornell Agr. Expt. Sta. Mem. 95, 21 p.



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## CHEMICAL COMPOSITION OF APPLE JUICES AS AFFECTED BY CLIMATIC CONDITIONS<sup>1</sup>

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### PURPOSE OF THE WORK

Students of climatology in its relation to the growth of crops have confined their studies to annual plants, and there are in the literature no reports of work in which perennials growing under controlled cultural conditions have been studied for long periods in an attempt to ascertain whether annual variations in climatic conditions affect the chemical composition of the fruit in a definite and consistent fashion. An earlier paper from this laboratory (11)<sup>2</sup> reports an investigation of this question, employing grapes as experimental material, in which it has been shown that the chemical composition of the fruit of a large group of varieties shows from year to year consistent and sustained response to the annual fluctuations in the intensity of certain climatic factors. The results are such as to suggest that the composition of the annual crop is a physiological response of the plant to climatic factors of the environment of such fundamental character that large groups of horticultural varieties of widely dissimilar character exhibit identical behavior. The present paper reports the result of an investigation begun concurrently with that upon grapes, in which a large collection of apples has been studied for a period of six years in an attempt to ascertain whether the composition of the fruit from year to year is in any degree a reflection of the climatic conditions prevailing during its development.

In order that an experiment of this character may be at all conclusive, certain conditions must be met at the outset. The non-climatic factors, such as physical and chemical character of soil, slope, and exposure, must be as uniform as possible, and there must be a past and present history of uniformity in treatment, cultural and otherwise, of the experimental material, and an assurance of the continuance of such likeness of treatment throughout the experiment. With these conditions assured, any experiment of this character gains greatly in value if the experimental material used be as widely varied in character as it is possible to procure, in order that a wide range of individual variations may be encountered. If a fundamental physiological response of the organism to environmental factors really occurs, it should break through all the minor phenomena resulting from causes inherent in or peculiar to individuals and manifest itself as mass behavior. The more heterogeneous the assemblage of individuals for such an experiment can be made, the more significance can be attached to any clear-cut evidence that the assemblage is acting as a unit.

<sup>1</sup> Received for publication July 28, 1926; issued April, 1928. This paper is the third in a series of studies on fruit juices. References to the earlier papers in the series are given in "Literature cited" (10, 11).

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 364.

## MATERIAL EMPLOYED

The collection of apple varieties maintained by the Office of Horticulture of the Bureau of Plant Industry at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., offers an exceptional opportunity for such a study. The collection contains about 600 varieties, there being two or occasionally four trees of a variety, most of the trees being 11 to 14 years of age at the time the work was begun. Most of the varieties which have attained commercial importance in any of the producing districts of the United States are represented, and in addition the collection has a very large number of the older sorts which have been restricted to home orchards or which have been displaced in commercial orchards. In addition, there is a considerable number of varieties of diverse origin under test by the Office of Foreign Plant Introduction and a number of French cider apples propagated from the collection brought from France by W. B. Alwood (4).

From this collection a list of 100 varieties was selected in 1919. The number was determined by necessity rather than by choice, since the time available for the analytical work was limited. In selecting the varieties to be used an attempt was made to include only trees apparently in good condition, free from evidence of serious disease, and with a history of regular bearing of crops of fairly uniform size. From the list of varieties meeting these requirements an attempt was made to select 100 which would represent the greatest possible range in dessert and culinary quality and in chemical composition. Preliminary analyses made in 1918 and 1919, published analyses, descriptions of varieties in horticultural literature, and information furnished by associates in the Office of Horticulture were utilized as guides in making up the final selections. Analyses were made upon the members of this list, in so far as they were in fruit, in 1920 and 1921. From the experience of these two years it was realized that for various reasons not more than 70 to 75 per cent of the group would be in fruit in any one year and that enlargement of the numbers employed was therefore desirable. As the analytical data to be obtained were necessary as a background for other investigations, in progress or projected (12), additional time was devoted to the work and the original list of 100 varieties was increased to 250 in 1921. It remained at that number for the last four years of the work. Not more than 202 of this number were analyzed in any one year, and 32 are not included in the analytical results for various reasons. Some proved to be irregular in bearing, or bloomed so early that frost injury was of frequent occurrence. Death of trees and disease or injury of one of the pair of trees of a variety necessitated the dropping of others, since the material for analysis was always a mixed sample taken from the two trees of the variety, and no sample was taken when only one tree of the variety was in fruit. These causes, with partial destruction of the crop by frost in two years and the nearly universal habit of apple trees of occasionally failing to fruit without discoverable reason, resulted in considerable irregularity in the number of varieties analyzed from year to year.

In the final tabulation of the results no variety of the original list of 100 is included unless it bore fruit in four of the six years of the test; of the 150 varieties added in 1922, none is included unless it fruited in three of the four years following its addition to the list.



The general results are consequently free from the error which would result from inclusion of data derived from occasional crops borne by varieties with the alternate bearing habit.

The following statement as to the orchard location, soil, cultural treatment, and general handling of the trees has been prepared by H. P. Gould, of the Office of Horticulture; who has had direct supervision of the orchard during the work.

#### THE VARIETY APPLE ORCHARD AT ARLINGTON EXPERIMENT FARM

The entire orchard at the Arlington Experiment Farm, excepting certain seedling trees in one section, consists of trees propagated at the farm as piece-root grafts and grown there during their nursery period. The stocks used were ordinary commercial French crab seedlings. The scions came from a great number of sources. The late W. N. Irwin, for many years a member of the pomological staff of the department, first in the Division of Pomology and later in the Office of Pomological Investigations, supplied scions of more varieties than were obtained from any other one source. These were taken from a private variety collection which he had assembled from many sources during a period of several years, and which he had propagated and grown practically under nursery conditions on a piece of land located in the outskirts of Washington. Scions of many varieties were obtained from the New York Experiment Station at Geneva. Others came from many and widely diverse sources. The trees were grown in the nursery at Arlington farm during two seasons in most if not all cases and were planted as 2-year-olds.

The site occupied by the orchard at the Arlington farm varies somewhat in topography. A portion is level in the main, while certain sections are described as rolling. In the latter the slopes in general are east and west, though in certain areas the aspect is north. However, the slopes are not sufficient to influence the orchard materially except as they may result in better soil and atmospheric drainage than would otherwise prevail. The times of blossoming and ripening are probably not influenced in any degree by the topography, as the circulation of air throughout the orchard is unimpeded and is influenced but little if any by the irregularities of contour.

The soil is naturally a rather heavy reddish clay with a clay subsoil. Most of the farm, presumably including also the site of the orchard, is underlain at a depth of 3 or 4 feet with a tight impervious "pipe clay." While comparatively little of the site suffers seriously from lack of soil drainage, due probably to its more or less rolling topography, there are certain areas where the water stands at times for a considerable period. A few of the trees have suffered as a result. The constant use of soil-improvement crops and the application of manure on the poorer areas have materially improved the physical condition of the surface, so that, although normally a rather stiff clay, it has become somewhat loamy because of the humus that has been added.

The oldest portion of the orchard was planted in the spring of 1905. Additional smaller plantings were made in 1906 and 1909 and at various times up to 1911.

The management of the orchard up to about 1915 followed in a routine way an ordinary farm orchard plan of maintenance that involved tillage, cover crops, spraying, and pruning. Stable manure has been used from time to time on some of the poorer spots. Since 1915 the same general plan of maintenance has been followed, but the system of pruning has been subject to considerable modification from time to time; at times little or no pruning has been done, at other times the pruning has been unwise and doubtless harmful in its effects.

Since about 1921 the orchard has been sprayed in accordance with good commercial orchard practice so far as the schedule of applications is concerned. In most seasons during this time the applications have been fairly effective, considering the inherent difficulties in spraying a variety orchard.

In the spring of 1924 the trees received phosphoric acid and nitrate of soda at the general rate of 5 pounds of each to the tree. In the spring of 1925 the trees were given 5 pounds of nitrate of soda only. Since 1918 the older part of the orchard has been in sod. The grass has been cut once or twice during the season and left on the ground where it fell. The rest of the orchard has been cultivated more or less, usually with some cover crop on the land during the season.

The general plan of operation in recent years has aimed to maintain the orchard as nearly as possible on the plan of the well-kept farm orchard, no attempt being made to stimulate the trees to maximum production.

## PHENOLOGICAL DATA

Detailed records as to dates of blooming of the varieties have not been regularly made during the period covered by the work here reported. Such records were taken in 1919, 1924, and 1925, but were not made in the intervening years. A fairly trustworthy estimate can be obtained from the records of the various orchard operations at the Arlington farm, which include a record of the dates at which the various spray applications were made. The calyx spray is usually applied when the petals have fallen from all but the latest blooming varieties, and the date upon which its application begins practically coincides with the end of the blooming period.

Thus in 1919 blooming was beginning April 17, was at its height April 22, and was practically over on April 30. Spraying was begun April 30 and continued through May 4. In 1920 no blooming records were kept, but spraying was begun on May 9, or 10 days later than in 1919. In 1921, 1922, and 1923 spraying was begun, respectively, on May 2, April 29, and April 30, indicating that the blooming period in these years was very nearly identical with that of 1919. In 1924 detailed records indicate that blooming began April 24, reached its height about May 3, and was practically over on May 8. Spraying was begun on May 9. In 1925 the blooming records give April 12 to 25 as the period in which the great majority of varieties came into bloom. Spraying was begun on April 25.

Summarizing, it appears to be a close approximation to the facts to say that in 1921, 1922, and 1923 the blooming period was largely included in the period April 15 to 30; that in 1925 it occurred about five days earlier, or from April 10 to 25; and that in 1920 and 1924 it was decidedly late, extending from about April 24 to May 8. The last half of April is probably the normal blooming period in this latitude for the great majority of the varieties present in the orchard, 1925 having been a year of early bloom while in 1920 and 1924 blooming was quite late. These seasonal variations in date of blooming are reflected in a broad general way in the dates of picking for the various years, which are in the majority of cases distinctly later in 1920 and 1924. The dates of picking of the samples are in all cases stated in the table giving the analytical data. (See Table 1.)

## METHODS OF TAKING SAMPLES

The fruit employed for analysis was in all cases a tree-run sample of the crop of the variety, taken at the time the variety was harvested. In the great majority of instances the entire crop of the pair of trees was picked at one time, but in some of the early-maturing varieties two or rarely three pickings were occasionally made. In these cases the sample was taken from the picking which included the bulk of the crop. From the unsorted tree-run fruit of each variety a quantity ranging from 2 to 10 bushels (the quantity depending upon the size of the crop and the demand for that variety for use in other work) was delivered to the laboratory immediately after picking and there stored until used. The stage of maturity attained at picking was that known among orchardists as "market ripe," as determined by the judgment of an experienced orchard foreman conversant with the varieties present in the orchard.

As received at the laboratory the fruit was stored in lug boxes in a concrete-walled basement room and there held until it had reached the condition of cider ripeness; that is, a condition midway between picking ripeness and dessert ripeness. Fruit in this stage has attained the full flavor and bouquet of the variety, has begun to soften very slightly, but is still too firm for eating out of hand. The temperatures in the storage room fluctuated with the outside temperature, remaining 8° to 10° F. below it, so that the time that fruit remained in storage prior to pressing varied with season, variety, and degree of ripeness when received, but is indicated in every case by inclusion in the analytical data of the dates of picking and of pressing.

It was usually the case toward the end of the apple harvest that a large number of varieties were picked practically at one time. In these cases the fruit was taken directly from the orchard into cold storage and there held until the end of the picking season. It was then transferred to the basement storage-room already mentioned, where it remained until it attained the proper condition for pressing.

That there should be some variation in the degree of maturity attained both at picking and at pressing as a result of errors in judgment is inevitable. It is believed that such errors are reduced to a minimum by the fact that the same individuals handled all samples at pressing throughout the work.

The entire lot of any variety was always pressed together, without discarding any specimens except those partially decayed. The samples were pressed without washing in order to avoid dilution of the juice, and a sufficient number of clean, dry press cloths were provided to permit of their use only once before washing and drying. In most of the work pressing was done in a power-operated hydraulic press, but a smaller hand-operated press was also used, mainly for smaller lots. The smaller press had been so modified as to make possible as good an extraction of juice as was obtained with the power press, and care was taken to assure such extraction.

All the juice from a variety was received in a large vessel, and samples for analysis were immediately taken, the juice being thoroughly stirred meanwhile to obtain uniformity. The analyses were begun immediately and were always completed on the same day the juice was pressed. In all cases samples of the juice were taken in triplicate or quadruplicate and preserved by Pasteurization. Most of these Pasteurized samples were subsequently analyzed in connection with the studies of the effect of Pasteurization in progress concurrently with the present work. The results of the second analyses incidentally serve as a general check upon the accuracy of the first, and have consequently made possible the omission from the tabulated results of a few analyses in which errors had obviously occurred.

#### ANALYTICAL METHODS

In 1920 and 1921 the only determinations made upon the fresh juices were those of titratable acidity, free reducing sugars, and total sugars after inversion. In 1922 and subsequently these were supplemented by determinations of total astringency and astringent non-tannins. Determinations of total solids in juices were occasionally but not regularly made.

Determinations of total titratable acidity were made by titration with N/10 sodium hydroxide against phenolphthalein as indicator, after dilution of the juice with 10 volumes of distilled water.

Determinations of free reducing sugars were made by the Munson and Walker method, employing neutral lead acetate for clarification, and potassium oxalate for removing the surplus lead. The cuprous oxide was dissolved in ferric ammonium sulphate and titrated with N/20  $\text{KMnO}_4$ .

Total sugars were determined upon a portion of the clarified solution by inverting with HCl and employing the same method as for reducing sugars.

Determinations of total astringency were made by the Loewenthal-Proctor method, titrating the diluted juice with N/20  $\text{KMnO}_4$  in presence of indigo carmine. Astringent nontannins were determined in the same way after removal of tannins by precipitation with gelatin and filtration with kaolin. The results are expressed in terms of the conventional tannin factor.

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 6 years:										
Baker.....	Sept. 14, 1920	Oct. 7	9.18	0.18	9.36	0.684	-----	-----	-----	-----
	Aug. 23, 1921	Sept. 27	8.96	2.44	11.40	.718	-----	-----	-----	-----
	Sept. 25, 1922	Nov. 15	7.88	4.08	11.96	.590	0.0823	0.0385	0.0438	15.32
	Sept. 24, 1923	Oct. 6	8.54	4.74	13.28	.419	.0961	.0386	.0575	16.13
	Oct. 1, 1924	Oct. 9	7.58	4.56	12.14	.555	.1122	.0360	.0762	-----
	Sept. 17, 1925	Oct. 6	6.48	3.49	9.97	.542	.0903	.0361	.0542	12.42
Celestia.....	Oct. 5, 1920	Oct. 18	8.76	2.13	10.89	.606	-----	-----	-----	13.09
	Aug. 18, 1921	Oct. 3	6.91	2.61	9.52	.585	-----	-----	-----	-----
	Sept. 27, 1922	Dec. 8	8.39	1.46	9.85	.551	.0918	.0169	.0749	13.11
	Sept. 16, 1923	Sept. 29	8.30	3.33	11.63	.588	.0650	.0150	.0500	13.63
	Oct. 14, 1924	Oct. 16	8.31	2.23	10.54	.353	.0660	.0180	.0480	-----
	Sept. 10, 1925	Sept. 23	9.40	2.56	11.96	.384	.0858	.0323	.0533	14.84
Collins.....	Oct. 15, 1920	Oct. 28	9.50	2.50	12.00	.650	-----	-----	-----	13.51
	Sept. 2, 1921	Sept. 19	8.64	2.43	11.07	.802	-----	-----	-----	-----
	Sept. 30, 1922	Nov. 1	7.52	1.54	9.06	.680	.0872	.0265	.0607	11.46
	Oct. 26, 1923	Oct. 31	7.35	5.15	12.50	.740	.1112	.0222	.0890	-----
	Oct. 28, 1924	Nov. 4	6.08	3.34	9.42	.517	.0995	.0295	.0700	-----
	Oct. 7, 1925	Nov. 23	7.82	2.56	10.38	.372	.1058	.0373	.0685	11.57
Indian.....	Oct. 15, 1920	Nov. 3	7.18	1.30	8.48	.522	-----	-----	-----	9.77
	Sept. 2, 1921	Oct. 8	8.96	2.99	11.95	.561	-----	-----	-----	-----
	Oct. 7, 1922	Oct. 24	7.70	2.35	10.05	.461	.1380	.0469	.0911	12.28
	Oct. 8, 1923	Oct. 18	8.08	3.00	11.08	.590	.1055	.0352	.0708	13.52
	Nov. 4, 1924	Nov. 13	8.58	2.44	11.02	.419	.1715	.0743	.0972	-----
	Oct. 16, 1925	Nov. 18	7.26	2.30	9.56	.318	.1380	.0440	.0940	11.05
Jeffers.....	Aug. 14, 1920	Sept. 21	8.12	1.09	9.21	.510	-----	-----	-----	11.25
	Aug. 1, 1921	Sept. 5	7.96	2.62	10.58	.607	-----	-----	-----	-----
	July 27, 1922	July 31	7.52	1.04	8.56	.808	.1481	.0418	.1063	10.69
	Aug. 23, 1923	Aug. 30	8.15	5.21	13.36	.642	.1165	.0568	.0597	14.87
	Aug. 29, 1924	Sept. 6	7.98	3.30	11.28	.342	.1050	.0735	.0315	-----
	Aug. 8, 1925	Aug. 15	8.24	3.53	11.77	.568	.1010	.0497	.0513	-----
Nero.....	Oct. 1, 1920	Oct. 20	9.01	2.19	11.20	.294	-----	-----	-----	14.32
	Aug. 24, 1921	Sept. 18	8.45	3.29	11.74	.460	-----	-----	-----	-----
	Sept. 30, 1922	Oct. 27	8.98	3.52	12.50	.292	.1123	.0238	.0885	13.67
	Oct. 26, 1923	Nov. 1	9.64	2.97	12.61	.363	.1320	.0590	.0730	15.80
	Oct. 28, 1924	Dec. 1	8.55	1.85	10.40	.285	.1255	.0247	.1008	-----
	Oct. 1, 1925	Oct. 29	7.67	2.25	9.92	.254	.1165	.0595	.0570	12.37



TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 5 years— Continued. Entz-----	Nov. 5, 1920	Nov. 29	10.88	1.49	12.37	1.037	-----	-----	-----	13.91
	Sept. 6, 1921	Oct. 30	7.64	1.50	9.14	1.080	-----	-----	-----	-----
	Sept. 18, 1922	Nov. 16	7.26	2.46	9.72	1.117	0.3460	0.2313	0.1147	16.04
	Sept. 5, 1923	Sept. 18	5.26	2.94	8.20	1.425	.2830	.1347	.1483	11.90
	Sept. 26, 1924	Oct. 14	6.06	3.21	9.27	1.375	.4130	.2010	.2120	-----
Evening Party---	Sept. 15, 1920	Oct. 6	9.48	1.62	11.10	.276	-----	-----	-----	13.83
	Sept. 2, 1921	Oct. 4	9.90	2.06	11.96	.308	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 7	6.90	3.12	10.02	.330	.0912	.0368	.0544	13.08
	Sept. 27, 1923	Nov. 2	10.16	4.02	14.18	.272	.1242	.0497	.0745	17.08
	Oct. 11, 1924	Oct. 14	7.41	3.28	10.69	.335	.1187	.0459	.0728	-----
Granny Smith---	Nov. 1, 1920	Nov. 13	10.68	1.81	12.49	.731	-----	-----	-----	13.90
	Sept. 2, 1921	Oct. 7	9.16	2.36	11.52	.708	-----	-----	-----	-----
	Oct. 26, 1922	Oct. 31	7.38	6.27	13.65	.644	.0947	.0275	.0672	14.97
	Nov. 8, 1923	Nov. 12	6.88	3.36	10.24	.520	.0942	.0480	.0462	12.64
	Oct. 28, 1924	Dec. 2	7.27	3.71	10.98	.691	.1150	.0550	.0600	-----
Hilare-----	Aug. 18, 1921	Oct. 16	8.79	5.18	13.97	.838	-----	-----	-----	-----
	Sept. 28, 1922	Dec. 8	9.70	1.34	11.04	.817	.1474	.0677	.0797	14.47
	Sept. 28, 1923	Oct. 9	9.13	2.71	11.84	.890	.1045	.0393	.0652	15.18
	Sept. 23, 1924	Oct. 4	8.53	2.13	10.66	.799	.1355	.0798	.0557	-----
	Sept. 25, 1925	Oct. 29	8.70	1.54	10.24	.437	.0912	.0401	.0511	12.93
Hubbardston---	Sept. 3, 1920	Sept. 27	9.68	1.52	11.20	.595	-----	-----	-----	-----
	Aug. 25, 1921	do-----	9.02	3.55	12.57	.480	-----	-----	-----	-----
	Sept. 18, 1922	Dec. 11	8.22	2.54	10.76	.598	.0949	.0422	.0527	13.47
	Sept. 27, 1923	Oct. 5	7.18	3.76	10.94	.410	.0746	.0241	.0505	12.75
	Oct. 1, 1925	Oct. 20	6.94	2.95	9.89	.168	.0878	.0377	.0501	11.74
Keeper-----	Sept. 24, 1921	Nov. 3	8.09	3.64	11.73	.650	-----	-----	-----	-----
	Oct. 26, 1922	Oct. 27	8.52	3.00	11.52	.545	.0584	.0151	.0433	13.20
	Nov. 8, 1923	Nov. 16	7.72	3.24	10.96	.383	.0497	.0157	.0342	12.02
	Nov. 4, 1924	Nov. 18	8.44	.98	9.42	.392	.0448	.0135	.0813	-----
	Oct. 10, 1925	Nov. 2	7.42	2.62	10.04	.380	.0495	.0095	.0400	12.07
Klickatat-----	Sept. 13, 1921	Oct. 20	8.68	4.04	12.72	.507	-----	-----	-----	-----
	Oct. 15, 1922	Oct. 21	7.32	2.93	10.25	.393	.0770	.0345	.0425	11.80
	Oct. 4, 1923	Nov. 22	8.43	2.91	11.34	.362	.0924	.0471	.0453	13.04
	Nov. 3, 1924	Nov. 4	6.86	3.06	9.92	.401	.0820	.0373	.0447	-----
	Oct. 3, 1925	Oct. 26	7.08	2.13	9.21	.290	.0750	.0325	.0425	-----
Kooroochiang---	Oct. 30, 1920	Nov. 6	7.13	1.81	8.94	.976	-----	-----	-----	11.58
	Oct. 13, 1922	Oct. 17	5.93	2.84	8.77	.850	.0614	.0202	.0412	-----
	Oct. 26, 1923	Oct. 19	6.10	4.35	10.45	.862	.0857	.0312	.0545	13.41
	Nov. 4, 1924	Dec. 1	6.06	3.36	9.42	.820	.0995	.0297	.0698	-----
	Oct. 10, 1925	Nov. 21	8.28	3.72	12.00	.567	.1035	.0400	.0635	13.61
Lawyer-----	Oct. 12, 1920	Oct. 30	6.26	1.46	7.72	.617	-----	-----	-----	-----
	Sept. 2, 1921	Oct. 21	8.20	3.43	11.63	.565	-----	-----	-----	-----
	Sept. 29, 1922	Dec. 13	7.77	2.37	10.14	.563	.0729	.0342	.0387	-----
	Sept. 27, 1923	Sept. 29	7.43	3.77	11.20	.694	.0940	.0248	.0692	12.15
	Sept. 15, 1924	Oct. 9	7.74	2.32	10.06	.500	.1260	.0582	.0678	-----
Martha-----	Aug. 4, 1920	Sept. 1	8.95	1.85	10.80	.983	-----	-----	-----	12.86
	July 30, 1921	Aug. 16	8.44	3.04	11.48	.904	-----	-----	-----	-----
	July 28, 1922	July 29	7.37	2.47	9.84	.930	.1931	.0869	.1062	11.08
	Aug. 14, 1923	Aug. 27	9.36	2.33	11.69	.850	.2320	.1293	.1027	14.41
	Aug. 6, 1925	Aug. 10	8.76	2.92	11.68	.824	.1940	.0925	.1015	-----
Martin-----	Aug. 24, 1921	Oct. 23	9.00	3.57	12.57	.415	-----	-----	-----	-----
	Sept. 30, 1922	Oct. 4	8.45	3.01	11.46	.291	.0851	.0347	.0504	13.43
	Oct. 20, 1923	Oct. 30	9.54	4.44	13.98	.228	.0881	.0385	.0496	15.53
	Oct. 11, 1924	Oct. 20	8.00	2.38	11.38	.252	.0704	.0218	.0486	-----
	Oct. 7, 1925	Oct. 29	10.82	2.44	13.26	.241	.1280	.0530	.0750	16.23
McAfee-----	Oct. 8, 1921	Oct. 26	7.86	3.05	10.91	.620	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 11	7.49	2.60	10.09	.507	.1078	.0499	.0579	13.01
	Oct. 13, 1923	Oct. 26	9.34	2.66	12.00	.394	.1035	.0428	.0607	13.60
	Oct. 21, 1924	Nov. 13	8.24	1.34	9.58	.375	.1132	.0422	.0710	-----
	Oct. 15, 1925	Nov. 16	8.56	1.68	10.24	.235	.1210	.0480	.0730	11.95

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							Total solids
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	
Fruiting 5 years-- Continued.										
McIntosh-----	Sept. 1, 1920	Sept. 16	8.13	1.66	9.79	0.423				
	Sept. 1, 1922	Sept. 5	8.03	1.39	9.42	.566	0.0966	0.0518	0.0448	11.36
	Sept. 5, 1923	Sept. 13	8.00	3.34	11.34	.646	.1248	.0616	.0632	13.44
	Sept. 13, 1924	Sept. 25	7.04	3.36	10.40	.419	.0717	.0294	.0423	
	Aug. 21, 1925	Sept. 8	6.66	2.75	9.41	.371	.1040	.0398	.0642	11.84
Monmouth-----	Oct. 7, 1920	Oct. 30	7.74	2.02	9.76	.602				11.85
	Sept. 18, 1922	Sept. 23	7.60	1.99	9.59	.543	.0677	.0286	.0391	12.68
	Oct. 20, 1923	Nov. 6	7.74	3.03	10.77	.386	.0857	.0300	.0557	13.24
	Oct. 21, 1924	do	7.06	.96	8.02	.413	.0955	.0287	.0668	
	Oct. 5, 1925	Nov. 16	9.32	1.75	11.07	.300	.0985	.0403	.0582	12.91
Mother-----	Sept. 1, 1920	Sept. 19	9.46	.57	10.03	.413				
	Aug. 30, 1922	Sept. 15	6.89	4.15	11.04	.347	.1034	.0313	.0721	13.21
	Sept. 8, 1923	Sept. 22	6.86	4.48	11.34	.422	.1095	.0420	.0675	12.50
	Aug. 29, 1924	Sept. 15	6.97	4.37	11.34	.487	.1215	.0717	.0498	
	Sept. 14, 1925	Sept. 22	8.24	4.02	12.26	.267	.1242	.0742	.0500	14.08
Peron-----	Aug. 6, 1921	Sept. 16	7.11	3.05	10.16	.668				
	Aug. 16, 1922	Aug. 18	6.36	4.42	10.78	1.055	.1921	.1022	.0899	12.49
	Aug. 23, 1923	Sept. 5	6.12	4.12	10.24	.550	.1212	.0631	.0581	11.59
	Sept. 5, 1924	Sept. 16	5.16	3.32	8.48	.458	.1260	.0860	.0400	
	Sept. 1, 1925	Sept. 8	5.56	4.06	9.62	.313	.1415	.0663	.0752	12.43
Pifer-----	Sept. 13, 1920	Oct. 6	6.70	1.17	7.87	.449				11.01
	Oct. 11, 1921	Oct. 25	7.68	3.70	11.38	.354				
	Oct. 19, 1922	Oct. 20	8.26	1.90	10.16	.317	.0858	.0318	.0540	12.49
	Oct. 20, 1923	Oct. 26	10.02	4.03	14.05	.447	.1112	.0502	.0610	16.26
	Nov. 4, 1924	Nov. 29	9.36	2.00	11.36	.289	.0995	.0405	.0590	
Pyrus angusti- folia.*	Oct. 11, 1921	Nov. 6	3.61	1.14	4.75	2.190	.5380	.3100	.2280	9.86
	Oct. 4, 1922	Oct. 4	2.98	.24	3.22	2.316	.6882	.3580	.3302	7.77
	Oct. 26, 1923	Nov. 23	3.94	1.61	5.55	2.004	.9350	.4960	.4390	10.40
	Nov. 1, 1924	Nov. 1	2.97	1.47	4.44	2.504	.6900	.3270	.3630	7.63
	Nov. 6, 1925	Nov. 21	2.94	1.40	4.34	2.140	.8350	.2930	.5420	8.13
Ralls-----	Sept. 2, 1921	Oct. 15	7.39	4.98	12.37	.680				
	Oct. 7, 1922	Oct. 12	7.81	3.07	10.88	.583	.0789	.0246	.0543	14.92
	Oct. 20, 1923	Oct. 26	8.32	3.37	11.69	.420	.0727	.0282	.0445	13.13
	Oct. 14, 1924	Oct. 23	7.62	2.89	10.51	.355	.0940	.0211	.0429	
	Oct. 10, 1925	Nov. 18	8.76	2.52	11.28	.286	.0950	.0368	.0582	13.31
Shackleford-----	Aug. 5, 1921	Sept. 15	6.16	3.76	9.92	.552				
	Sept. 6, 1922	Sept. 19	7.30	2.38	9.68	.535	.0695	.0261	.0434	11.64
	Sept. 27, 1923	Oct. 10	7.50	4.13	11.63	.480	.0945	.0335	.0610	14.61
	Oct. 14, 1924	Oct. 16	7.00	2.14	9.14	.423	.0977	.0394	.0563	
	Sept. 25, 1925	Oct. 13	7.48	3.45	10.93	.260	.1087	.0487	.0600	12.75
Shone-----	Sept. 2, 1921	Sept. 15	7.29	4.11	11.40	.645				
	Sept. 23, 1922	Dec. 7	8.81	2.62	11.43	.500	.0478	.0319	.0159	12.98
	Oct. 26, 1923	Nov. 2	8.00	4.24	12.24	.576	.0700	.0297	.0403	13.62
	Oct. 11, 1924	Oct. 22	7.10	3.20	10.30	.467	.0662	.0165	.0467	
	Oct. 3, 1925	Oct. 31	8.78	2.72	11.50	.374	.0638	.0188	.0450	13.77
Smokehouse-----	Sept. 3, 1921	Sept. 26	9.07	3.81	12.88	.672				
	Sept. 28, 1922	Oct. 13	6.77	4.12	10.89	.558	.0747	.0204	.0543	12.09
	Oct. 4, 1923	Nov. 14	9.02	4.06	13.08	.559	.1200	.0472	.0728	15.70
	Sept. 13, 1924	Oct. 8	7.00	1.80	8.80	.440	.1020	.0412	.0608	11.20
	Oct. 3, 1925	Oct. 22	9.60	2.35	11.95	.378	.1225	.0457	.0768	15.12
Soulard-----	Nov. 1, 1920	Nov. 18	7.34	2.14	9.48	.897				12.15
	Oct. 11, 1921	Nov. 2	6.02	2.12	8.14	.861				
	Oct. 16, 1922	Oct. 18	5.90	1.39	7.29	.761	.1980	.1305	.0675	11.00
	Oct. 4, 1923	Dec. 6	5.28	1.71	6.99	.616	.2860	.1835	.1025	10.16
	Nov. 3, 1925	Nov. 25	6.18	2.36	8.54	.633	.3420	.1965	.1455	11.15
Sutton-----	Oct. 15, 1920	Nov. 11	6.72	3.20	9.92	.386				
	Sept. 23, 1922	Nov. 3	7.36	2.78	10.14	.456	.1071	.0354	.0717	12.71
	Sept. 23, 1923	Oct. 5	7.58	4.92	12.50	.615	.0815	.0267	.0548	13.37
	Sept. 24, 1924	Oct. 8	11.94	1.76	13.70	.585	.0960	.0368	.0592	
	Oct. 1, 1925	Oct. 6	7.18	5.38	12.56	.884	.1170	.0470	.0700	15.52

See footnote at end of table.

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							Total solids
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astringency	Tannin	Non-tannin astringency	
Fruiting 5 years—Continued. Swaar.....	Oct. 8, 1920	Oct. 30	10.13	3.77	13.90	0.612				14.66
	Sept. 23, 1922	Oct. 31	8.07	3.71	11.78	.598	0.1115	0.0398	0.0717	14.97
	Sept. 23, 1923	Oct. 8	8.14	4.64	12.78	.587	.0832	.0276	.0556	15.12
	Oct. 11, 1924	Oct. 22	9.03	3.64	12.67	.762	.1280	.0525	.0755	14.92
	Oct. 1, 1925	Oct. 23	8.06	4.42	12.48	.437	.0705	.0135	.0570	14.92
Sweet Orange....	Sept. 24, 1921	Oct. 17	8.92	3.80	12.72	.404				14.72
	Oct. 25, 1922	Oct. 30	7.33	6.54	13.87	.152	.0973	.0318	.0655	14.79
	Oct. 12, 1923	Oct. 26	10.63	3.21	13.84	.420	.0935	.0403	.0532	14.79
	Nov. 4, 1924	Nov. 11	10.48	2.64	13.12	.136	.0702	.0271	.0431	14.79
	Oct. 13, 1925	Nov. 2	10.16	3.65	13.81	.166	.1110	.0370	.0740	16.33
Titus.....	Oct. 8, 1920	Oct. 24	8.06	1.89	9.95	.535				13.98
	Aug. 25, 1921	Oct. 30	8.30	3.10	11.40	.407				13.98
	Sept. 23, 1922	Nov. 13	7.52	2.80	10.32	.279	.1717	.0933	.0784	13.14
	Sept. 28, 1923	Oct. 2	7.32	2.87	10.19	.582	.1132	.0557	.0625	13.14
	Oct. 1, 1925	Oct. 26	9.32	1.92	11.24	.314	.1390	.0630	.0760	14.74
Tompkins King..	Sept. 16, 1920	Oct. 16	7.94	2.06	10.00	.537				13.07
	Sept. 23, 1922	Dec. 11	8.71	1.74	10.45	.542	.0984	.0501	.0483	15.93
	Sept. 28, 1923	Oct. 8	9.48	3.25	12.73	.410	.1123	.0428	.0695	13.65
	Sept. 24, 1924	Oct. 3	7.23	2.75	9.98	.423	.1030	.0342	.0688	13.65
	Sept. 15, 1925	Oct. 19	7.26	4.07	11.33	.350	.1075	.0407	.0668	13.65
Transcendent....	Aug. 6, 1920	Aug. 24	7.96	2.42	10.38	.912				11.84
	Aug. 1, 1922	Aug. 7	8.32	1.66	9.98	1.140	.3617	.1696	.1921	14.44
	Aug. 20, 1923	Aug. 27	7.44	4.27	11.71	.783	.2530	.1415	.1115	14.44
	Aug. 18, 1924	Aug. 21	7.64	1.16	8.80	.417	.3180	.1230	.1950	14.47
	Aug. 8, 1925	Aug. 10	6.41	3.68	10.09	.776	.2136	.1026	.1110	14.47
Vanderpool.....	Aug. 31, 1921	Oct. 1	7.43	4.65	12.08	.503				15.67
	Sept. 23, 1922	Dec. 12	8.96	4.14	13.10	.391	.0862	.0393	.0469	15.71
	Sept. 16, 1923	Oct. 1	8.24	4.64	12.88	.535	.1476	.0711	.0765	15.71
	Oct. 4, 1924	Oct. 21	10.70	4.26	14.96	.397	.1295	.0525	.0770	14.47
	Sept. 16, 1925	Oct. 29	9.84	2.33	12.17	.260	.1280	.0600	.0680	14.47
Vandevere.....	Oct. 6, 1921	Oct. 24	9.86	2.06	11.92	.480				12.07
	Sept. 25, 1922	Oct. 13	6.78	4.11	10.89	.553	.0745	.0202	.0543	16.96
	Oct. 12, 1923	Oct. 23	9.12	5.41	14.53	.612	.1321	.0455	.0866	12.06
	Oct. 8, 1924	Oct. 20	7.96	4.30	12.26	.560	.1080	.0515	.0565	12.06
	Sept. 17, 1925	Nov. 16	7.12	3.31	10.43	.308	.0703	.0215	.0488	12.06
Westfield.....	Sept. 6, 1920	Oct. 17	9.22	2.30	11.52	.482				12.96
	Oct. 3, 1922	Oct. 7	7.33	3.03	10.36	.456	.1113	.0561	.0352	15.42
	Oct. 26, 1923	Nov. 2	8.00	5.87	13.87	.525	.1157	.0429	.0728	14.95
	Oct. 14, 1924	Oct. 17	7.86	2.34	10.20	.437	.0840	.0230	.0610	14.95
	Oct. 7, 1925	Oct. 16	7.24	4.94	12.18	.415	.1200	.0390	.0810	14.95
White Doctor....	Sept. 3, 1920	Oct. 16	7.50	2.10	9.60	.590				11.54
	Aug. 16, 1921	Oct. 7	7.33	2.35	9.68	.303				15.04
	Aug. 24, 1922	Oct. 4	6.32	2.74	9.06	.672	.0982	.0400	.0582	11.54
	Sept. 27, 1923	Nov. 21	8.41	2.98	11.39	.507	.0852	.0262	.0590	15.04
	Sept. 13, 1924	Sept. 26	5.82	2.66	8.48	.650	.0936	.0347	.0589	11.12
White Pippin....	Oct. 9, 1920	Oct. 20	8.68	1.01	9.69	.630				13.42
	Sept. 11, 1921	Oct. 13	8.40	3.08	11.48	.651				13.42
	Oct. 13, 1922	Oct. 19	6.34	4.70	11.04	.522	.0717	.0275	.0442	13.42
	Sept. 28, 1923	Nov. 14	6.62	4.77	11.39	.470	.0995	.0430	.0565	13.42
	Oct. 4, 1924	Oct. 9	6.04	4.02	10.06	.595	.0950	.0308	.0642	13.42
Wolf River.....	Sept. 1, 1920	Oct. 2	7.39	1.38	8.77	.520				10.18
	Aug. 19, 1922	Aug. 25	5.68	1.96	7.64	.786	.0766	.0235	.0531	13.22
	Sept. 27, 1923	Sept. 29	7.87	1.81	9.68	.740	.0940	.0352	.0588	11.17
	Sept. 15, 1924	Sept. 20	5.76	1.86	7.62	.559	.0765	.0342	.0423	11.17
	Sept. 16, 1925	Sept. 23	5.70	3.01	8.71	.560	.0750	.0333	.0417	11.17
Yellow Bellflower	Oct. 13, 1920	Nov. 2	7.86	2.16	10.02	.636				13.82
	Sept. 2, 1921	Nov. 6	9.00	3.77	12.77	.595				13.82
	Oct. 3, 1922	Oct. 5	7.36	3.17	10.53	.571	.0685	.0324	.0561	16.02
	Oct. 26, 1923	Oct. 31	9.40	5.56	14.96	.635	.1346	.0465	.0881	16.02
	Sept. 16, 1925	Oct. 6	6.78	3.43	10.19	.424	.0953	.0343	.0610	16.02



TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astrin-gency	Tan-nin	Non-tannin astrin-gency	Total solids
Fruiting 4 years:										
Abernathy.....	Aug. 23, 1922	Aug. 28	8.70	0.44	9.14	0.421	0.0940	0.0250	0.0690	11.85
	Aug. 29, 1923	Dec. 12	8.90	2.04	10.94	.395	.1652	.0860	.0792	12.49
	Sept. 15, 1924	Sept. 25	7.18	2.66	9.84	.339	.0813	.0358	.0455	-----
	Aug. 30, 1925	Sept. 14	7.84	2.32	10.16	.294	.1150	.0516	.0634	12.48
Algérienne.....	Aug. 19, 1922	Aug. 28	5.93	4.07	10.00	.383	.2761	.1104	.1657	13.60
	Sept. 13, 1923	Sept. 21	5.97	5.58	11.55	.221	.2500	.1315	.1185	12.97
	Aug. 16, 1924	Aug. 24	5.02	4.24	9.26	.231	.1930	.1008	.0922	-----
	Oct. 7, 1925	Oct. 13	8.28	3.66	11.94	.150	.1570	.0812	.0758	14.29
Allington Pippin <sup>b</sup>	Aug. 29, 1922	Sept. 13	8.47	3.14	11.61	.946	.1206	.0460	.0746	12.66
	Sept. 24, 1923	Oct. 1	8.42	2.15	10.57	.585	.1290	.0580	.0710	12.53
	Sept. 13, 1924	Sept. 27	6.84	1.50	8.34	.615	.0892	.0446	.0446	-----
	Sept. 15, 1925	Nov. 7	6.65	2.55	9.20	.338	.1065	.0507	.0558	11.07
Amère du Sur-ville.	Sept. 23, 1922	Dec. 14	8.98	.94	9.92	.410	.3384	.2136	.1248	13.91
	Sept. 4, 1923	Sept. 19	9.70	1.64	11.34	.248	.7400	.4220	.3180	13.21
	Oct. 18, 1924	Nov. 8	8.97	2.01	10.98	.148	.4360	.2795	.1565	-----
	Oct. 10, 1925	Oct. 17	11.06	1.82	12.88	.176	.4740	.2140	.2600	15.87
Arctic.....	Sept. 28, 1922	Dec. 8	8.26	2.15	10.41	.439	.1155	.0358	.0797	-----
	Sept. 16, 1923	Nov. 15	9.13	4.84	13.97	.608	.1648	.0622	.1026	16.76
	Sept. 21, 1924	Oct. 2	6.72	3.38	10.10	.525	.1660	.0760	.0900	-----
	Sept. 10, 1925	Sept. 26	8.62	1.84	10.46	.360	.1265	.0518	.0747	13.33
Arnold.....	Aug. 30, 1921	Sept. 16	9.27	2.36	11.63	.776	-----	-----	-----	-----
	Sept. 1, 1922	Sept. 5	9.30	2.70	12.00	.583	.0949	.0181	.0768	14.03
	Sept. 16, 1923	Sept. 26	7.44	4.08	11.52	.617	.0638	.0394	.0544	12.89
	Sept. 13, 1924	Sept. 24	7.98	2.60	10.58	.480	.0690	.0549	.0441	-----
Babbitt.....	Aug. 29, 1922	Sept. 14	7.04	2.96	10.00	1.182	.1095	.0348	.0747	12.70
	Sept. 27, 1923	Sept. 29	7.44	3.26	10.70	1.180	.0957	.0317	.0640	12.98
	Sept. 13, 1924	Oct. 8	7.64	2.92	10.56	1.112	.1185	.0370	.0815	-----
	Sept. 17, 1925	Oct. 7	8.44	2.01	10.45	.910	.1002	.0337	.0665	13.54
Ben Hur.....	Oct. 6, 1922	Oct. 10	7.12	3.21	10.33	.368	.0763	.0211	.0552	12.94
	Oct. 9, 1923	Oct. 16	7.88	3.72	11.60	.373	.0778	.0281	.0497	14.16
	Nov. 4, 1924	Nov. 11	8.36	2.98	11.34	.312	.0650	.0228	.0422	-----
	Oct. 10, 1925	Nov. 2	7.35	2.99	10.34	.196	.0832	.0357	.0475	12.49
Bennet.....	Oct. 7, 1922	Oct. 17	7.24	5.64	12.88	.444	.0894	.0298	.0596	14.34
	Oct. 12, 1923	Oct. 20	8.16	4.90	13.06	.385	.0915	.0298	.0617	14.04
	Oct. 4, 1924	Dec. 3	6.92	2.18	9.10	.285	.0810	.0464	.0346	-----
	Oct. 8, 1925	Nov. 7	7.80	2.42	10.22	.312	.0977	.0423	.0554	12.01
Bethlehemite....	Aug. 19, 1922	Sept. 19	10.13	2.40	12.53	.350	.0799	.0365	.0434	14.53
	Sept. 27, 1923	Oct. 9	9.54	2.30	11.84	.385	.0945	.0379	.0566	14.53
	Sept. 24, 1924	Oct. 8	8.72	2.32	11.04	.349	.1015	.0440	.0575	-----
	Oct. 1, 1925	Oct. 13	8.58	1.34	9.92	.150	.0952	.0451	.0501	11.88
Black Annette....	Oct. 15, 1921	Oct. 30	8.67	4.27	12.94	.555	-----	-----	-----	-----
	Oct. 20, 1922	Oct. 25	6.54	3.94	10.48	.418	.1000	.0280	.0720	13.32
	Oct. 20, 1923	Oct. 27	7.48	5.82	13.30	.615	.1035	.0358	.0677	15.97
	Nov. 4, 1924	Dec. 2	6.82	3.24	10.06	.368	.1072	.0312	.0760	-----
Boiken.....	Sept. 14, 1920	Sept. 27	6.86	2.10	8.96	.830	-----	-----	-----	-----
	Sept. 23, 1922	Oct. 19	6.50	3.98	10.48	.811	.0672	.0274	.0398	11.08
	Sept. 19, 1923	Nov. 30	7.04	3.23	10.27	.589	.0762	.0335	.0427	11.96
	Oct. 8, 1924	Oct. 20	7.00	2.43	9.43	.784	.0635	.0301	.0334	-----
Camak.....	Sept. 23, 1921	Oct. 23	8.16	2.56	10.72	.396	-----	-----	-----	-----
	Sept. 30, 1922	Oct. 3	6.50	4.26	10.76	.423	.0843	.0261	.0582	13.20
	Oct. 20, 1923	Oct. 29	7.26	3.76	11.02	.272	.0917	.0489	.0428	11.94
	Sept. 25, 1925	Oct. 19	7.26	2.17	9.43	.227	.1000	.0432	.0568	11.81
Canada Reinette.	Sept. 22, 1921	Oct. 26	8.20	1.99	10.19	.430	-----	-----	-----	11.68
	Sept. 23, 1922	Dec. 5	8.80	2.94	11.74	.634	.1219	.0701	.0518	15.50
	Sept. 27, 1923	Oct. 4	7.88	4.79	12.17	1.010	.1270	.0445	.0825	16.69
	Sept. 15, 1924	Oct. 6	7.10	3.08	10.18	.710	.1515	.0805	.0710	-----

See footnote at end of table.

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astrin-gency	Tan-nin	Non-tannin astrin-gency	Total solids
Fruiting 4 years— Continued Carson.....	Sept. 6, 1922	Nov. 6	6.98	2.72	9.70	0.583	0.1239	0.0460	0.0779	11.76
	Sept. 19, 1923	Sept. 26	6.26	3.21	9.47	.595	.0658	.0080	.0678	11.72
	Sept. 7, 1924	Sept. 22	5.24	3.86	9.10	.752	.0540	.0165	.0375	-----
	Sept. 16, 1925	Sept. 25	8.10	2.67	10.77	.530	.0610	.0186	.0424	13.01
Cathine.....	Aug. 24, 1921	Sept. 20	7.12	2.68	9.80	.577	-----	-----	-----	-----
	Sept. 6, 1922	Oct. 17	7.14	3.44	10.58	.507	.1157	.0789	.0368	13.49
	Sept. 16, 1923	Oct. 2	8.14	3.86	12.00	.495	.1010	.0385	.0625	14.27
	Oct. 1, 1925	Oct. 17	7.94	2.65	10.59	.372	.1125	.0482	.0643	13.54
Clayton.....	Oct. 14, 1920	Nov. 3	9.12	3.45	12.57	.458	-----	-----	-----	13.86
	Sept. 23, 1922	Nov. 15	9.79	2.67	12.46	.349	.1103	.0420	.0683	-----
	Sept. 13, 1923	Sept. 28	9.14	3.52	12.66	.773	.1070	.0360	.0710	15.64
	Oct. 11, 1924	Oct. 22	8.19	2.77	10.96	.370	.1140	.0508	.0632	-----
Cox No. 12.....	Aug. 21, 1921	Sept. 13	7.38	3.15	10.53	.353	-----	-----	-----	-----
	Sept. 18, 1922	Sept. 21	7.00	3.96	10.96	.391	.0695	.0070	.0625	13.38
	Sept. 19, 1923	Sept. 24	7.44	2.67	10.11	.293	.0870	.0316	.0554	12.91
	Sept. 16, 1925	Oct. 5	7.38	1.64	9.02	.156	.0920	.0395	.0525	10.90
Cox No. 13.....	Sept. 18, 1922	Dec. 7	9.20	1.82	11.02	.508	.0504	.0163	.0341	14.69
	Sept. 19, 1923	Sept. 26	7.22	3.64	10.86	.530	.0640	.0150	.0490	12.37
	Oct. 21, 1924	Oct. 23	7.03	2.78	9.81	.376	.0590	.0170	.0420	11.64
	Sept. 16, 1925	Oct. 5	7.36	1.72	9.08	.269	.0584	.0190	.0394	11.03
Delicious.....	Sept. 25, 1920	Oct. 6	9.55	1.05	10.60	.254	-----	-----	-----	-----
	Sept. 21, 1921	Oct. 18	7.39	4.30	11.69	.313	-----	-----	-----	-----
	Oct. 7, 1922	Oct. 11	7.28	2.82	10.10	.279	.0808	.0377	.0491	12.46
	Oct. 14, 1924	Nov. 4	8.24	1.98	10.22	.216	.0760	.0322	.0438	-----
Dickey.....	Oct. 1, 1920	Oct. 27	7.66	2.09	9.75	.610	-----	-----	-----	12.93
	Oct. 20, 1923	Oct. 30	8.74	4.49	13.23	.412	.0908	.0291	.0617	14.97
	Oct. 11, 1924	Oct. 21	7.68	3.34	11.02	.390	.0910	.0430	.0480	-----
	Oct. 1, 1925	Oct. 20	7.86	3.23	11.09	.346	.1042	.0384	.0658	14.56
Dixon.....	Sept. 23, 1922	Nov. 8	8.20	2.49	10.69	.368	.0876	.0460	.0416	13.76
	Oct. 8, 1923	Oct. 13	7.66	1.82	9.48	.582	.0703	.0163	.0540	12.35
	Oct. 8, 1924	Oct. 22	8.36	3.12	11.48	.615	.0590	.0100	.0490	-----
	Sept. 23, 1925	Oct. 26	7.10	1.79	8.89	.372	.0620	.0186	.0434	-----
Domine.....	Oct. 7, 1922	Oct. 11	6.78	3.15	9.93	.426	.0947	.0711	.0236	12.83
	Oct. 8, 1923	Nov. 19	8.55	4.43	12.98	.514	.1110	.0606	.0504	12.10
	Oct. 11, 1924	Oct. 21	7.13	2.71	9.84	.443	.0635	.0267	.0368	-----
	Sept. 17, 1925	Oct. 8	8.70	3.19	11.89	.372	.0920	.0378	.0542	14.84
Dudley.....	Aug. 17, 1920	Sept. 2	8.40	1.78	10.18	.752	-----	-----	-----	-----
	July 23, 1922	July 28	6.21	3.15	9.36	1.093	.1369	.0245	.1124	11.98
	Aug. 4, 1923	Aug. 13	5.90	2.30	8.20	.762	.1223	.0478	.0745	10.58
	Sept. 5, 1924	Sept. 12	5.08	2.58	7.66	.545	.0791	.0351	.0440	-----
Dulaney.....	Sept. 13, 1921	Sept. 26	9.03	2.33	11.36	.531	-----	-----	-----	-----
	Oct. 13, 1922	Oct. 16	9.10	1.67	10.77	.355	.0894	.0429	.0465	12.94
	Oct. 20, 1923	Oct. 29	9.95	2.55	12.50	.408	.0925	.0368	.0557	14.02
	Oct. 10, 1925	Nov. 18	8.66	1.45	10.11	.215	.1175	.0540	.0635	12.71
Early Cooper....	July 19, 1921	Aug. 8	6.16	2.51	8.67	.586	-----	-----	-----	-----
	July 3, 1923	Aug. 6	7.06	.92	7.98	.570	.1110	.0512	.0598	9.19
	Aug. 18, 1924	Aug. 27	8.32	.94	9.26	.525	.0834	.0563	.0271	-----
	Aug. 4, 1925	Aug. 12	7.96	1.62	9.58	.404	.1108	.0551	.0557	-----
Early Ripe.....	July 14, 1920	July 17	6.32	1.06	7.38	.887	-----	-----	-----	-----
	July 10, 1922	July 10	5.76	1.52	7.28	.691	.1216	.0552	.0664	10.64
	July 13, 1923	July 23	7.54	2.60	10.14	.921	.1085	.0700	.0385	-----
	July 24, 1924	Aug. 4	6.00	.16	6.16	.683	.0895	.0308	.0587	-----
Fallawater.....	Sept. 18, 1920	Oct. 20	9.63	1.14	10.77	.374	-----	-----	-----	12.16
	Sept. 18, 1922	Oct. 31	9.26	-----	9.26	.221	.1124	.0355	.0769	12.23
	Sept. 27, 1923	Oct. 4	8.61	3.42	12.03	.418	.0780	.0162	.0618	15.42
	Oct. 8, 1924	Oct. 11	7.53	2.24	9.77	.256	.0832	.0316	.0516	-----

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 4 years— Continued. Fameuse.....	Sept. 12, 1920	Sept. 27	10.00	1.26	11.26	0.420				12.71
	Sept. 27, 1923	Oct. 4	8.98	3.05	12.03	.580	0.0960	0.0308	0.0652	15.45
	Sept. 15, 1924	Sept. 25	7.12	2.32	9.44	.454	.0900	.0362	.0338	
	Sept. 15, 1925	Sept. 23	8.80	2.48	11.28	.325	.0996	.0454	.0542	13.95
Florence.....	Aug. 23, 1921	Aug. 30	7.96	.36	8.32	1.105				
	July 24, 1922	July 28	6.29	1.19	7.48	.836	.3229	.1441	.1788	9.30
	Aug. 9, 1924	Aug. 27	7.06	.59	7.65	.639	.2900	.1404	.1496	
	July 29, 1925	Aug. 18	8.52	.60	9.12	.620	.1940	.0942	.0998	
Flory.....	Aug. 19, 1922	Aug. 28	5.35	3.42	8.77	.847	.1044	.0181	.0863	11.38
	Sept. 21, 1923	Sept. 26	5.58	3.30	8.88	.654	.0850	.0315	.0535	11.07
	Sept. 13, 1924	do.	4.78	2.54	7.62	.497	.1140	.0535	.0605	
	Sept. 9, 1925	Sept. 14	6.16	2.46	8.62	.505	.0808	.0192	.0616	11.02
Gold Medal.....	Oct. 8, 1920	Oct. 19	9.12	2.24	11.36	.520				12.97
	Oct. 7, 1922	do.	8.51	1.89	10.40	.241	.0885	.0460	.0425	11.63
	Oct. 8, 1924	Oct. 16	9.01	2.65	11.66	.318	.1090	.0595	.0495	
	Oct. 16, 1925	Nov. 23	9.32	3.24	12.56	.163	.1120	.0580	.0540	13.46
Golden Russet...	Oct. 8, 1920	Oct. 26	10.28	.39	10.67	.478	.0900	.0332	.0568	12.39
	Sept. 23, 1922	Nov. 17	11.01	1.33	12.34	.476	.0860	.0223	.0637	
	Sept. 28, 1923	Oct. 5	9.43	3.55	12.98	.605	.0961	.0386	.0575	15.46
	Oct. 8, 1924	Oct. 21	9.00	2.82	11.82	.447	.0800	.0303	.0497	
Greenville.....	Sept. 28, 1922	Nov. 3	6.62	3.51	10.13	.438	.1097	.0478	.0619	12.89
	Sept. 15, 1923	Sept. 29	6.66	4.27	10.93	.675	.0825	.0370	.0455	12.20
	Sept. 24, 1924	Oct. 4	5.38	4.74	10.12	.667	.0942	.0445	.0497	
	Oct. 1, 1925	Oct. 16	6.92	3.59	10.51	.422	.1045	.0460	.0555	13.10
Haas.....	Aug. 19, 1922	Aug. 21	8.71	2.67	11.38	1.050	.1191	.0255	.0936	13.26
	Sept. 16, 1923	Dec. 6	7.26	2.15	9.41	.445	.0852	.0272	.0580	14.30
	Sept. 5, 1924	Sept. 19	7.05	1.57	8.62	.583	.1170	.0410	.0760	
	Sept. 14, 1925	Sept. 22	7.72	3.11	10.83	.477	.1320	.0500	.0820	13.84
Hagloe Crab.....	July 26, 1922	July 28	6.23	1.26	7.49	1.104	.1359	.0123	.1236	9.86
	Sept. 3, 1923	Sept. 11	6.49	1.81	8.30	.484	.0930	.0430	.0500	8.86
	Sept. 5, 1924	Sept. 12	6.24	.84	7.08	.494	.1025	.0495	.0530	
	Aug. 21, 1925	Sept. 3	5.58	2.08	7.66	.449	.0965	.0425	.0540	10.94
Hoover.....	Sept. 1, 1922	Sept. 19	8.02	1.57	9.59	.604	.0808	.0243	.0565	11.28
	Sept. 8, 1923	Oct. 2	9.02	1.75	10.77	.676	.0942	.0368	.0574	13.29
	Sept. 13, 1924	Sept. 27	6.69	2.25	8.94	.561	.1245	.0641	.0604	
	Sept. 25, 1925	Oct. 19	8.84	1.51	10.35	.417	.0901	.0291	.0610	12.69
Huntsman.....	Oct. 13, 1922	Nov. 3	6.57	4.13	10.70	.279	.0982	.0292	.0690	11.84
	Sept. 28, 1923	Oct. 8	7.18	5.14	12.32	.476	.0763	.0216	.0547	13.97
	Oct. 8, 1924	Oct. 15	6.98	3.90	10.88	.502	.0832	.0154	.0678	
	Oct. 1, 1925	Oct. 26	6.66	2.48	9.14	.260	.0859	.0309	.0550	11.16
Hyslop.....	Aug. 29, 1922	Sept. 13	5.54	3.45	9.02	.583	.3284	.2077	.1207	11.62
	Sept. 8, 1923	Sept. 18	4.38	7.03	11.41	.517	.3180	.1768	.1412	12.79
	Sept. 13, 1924	Sept. 22	3.37	6.21	9.58	.567	.3700	.2390	.1310	
	Sept. 5, 1925	Sept. 14	3.44	5.92	9.36	.358	.2180	.1345	.0835	13.16
Hort. No. 3050 c.	Oct. 11, 1921	Nov. 4	10.16	1.76	11.92	.454				
	Nov. 3, 1923	Nov. 6	8.79	2.92	11.71	.408	.1440	.0610	.0830	15.08
	Nov. 2, 1924	do.	9.10	1.68	10.88	.437	.1320	.0565	.0755	
	Oct. 16, 1925	Oct. 31	9.68	1.76	11.44	.242	.1260	.0495	.0765	14.70
Hort. No. 4941 d.	Oct. 25, 1922	Oct. 30	9.06	3.84	12.90	.182	.1486	.0531	.0955	15.51
	Oct. 12, 1923	Oct. 26	8.46	3.17	11.63	.260	.1122	.0437	.0685	13.87
	Nov. 4, 1924	Nov. 18	9.24	4.12	13.36	.156	.1235	.0500	.0735	
	Oct. 15, 1925	Nov. 24	7.86	4.25	12.11	.150	.1283	.0443	.0840	13.97
Ingram.....	Sept. 13, 1921	Sept. 26	8.20	3.16	11.36	.442				
	Sept. 23, 1922	Nov. 15	7.46	3.30	10.76	.412	.1208	.0551	.0657	13.46
	Oct. 8, 1923	Oct. 12	8.00	2.77	10.77	.443	.0727	.0265	.0462	12.49
	Oct. 7, 1925	Nov. 23	7.92	3.36	11.28	.332	.1205	.0495	.0710	13.31

See footnotes at end of table.

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astringency	Tannin	Non-tannin astringency	Total solids
Fruiting 4 years—Continued. Jersey Sweet	Aug. 17, 1921	Sept. 16	8.30	2.47	10.77	0.118				
	Aug. 3, 1922	Aug. 4	7.61	3.85	11.46	.190	0.1645	0.0603	0.1042	13.26
	July 29, 1923	Aug. 7	5.44	3.71	9.15	.104	.1210	.0552	.0658	11.31
	Aug. 29, 1924	Sept. 2	6.91	3.59	10.50	.082	.0872	.0184	.0688	-----
Kentucky Sweet	Aug. 23, 1922	Aug. 28	8.89	1.00	9.89	.269	.2002	.1243	.0759	13.59
	Aug. 27, 1923	Sept. 12	8.31	3.33	11.64	.179	.1960	.0960	.1000	12.63
	Sept. 13, 1924	Sept. 27	7.66	2.38	10.04	.154	.1275	.0555	.0720	-----
	Sept. 1, 1925	Sept. 3	6.74	2.14	8.88	.149	.1180	.0580	.0600	12.54
Kinnard	Sept. 6, 1921	Oct. 8	6.34	4.44	10.78	.481	.0893	.0253	.0640	-----
	Sept. 28, 1923	Nov. 5	7.82	3.94	11.76	.435	.1080	.0439	.0641	13.66
	Oct. 8, 1924	Oct. 22	6.64	3.38	10.02	.470	.1045	.0473	.0572	-----
	Oct. 15, 1925	Oct. 31	7.54	2.09	9.63	.314	.0899	.0302	.0567	12.19
Lou	July 24, 1920	Aug. 6	7.32	.32	7.64	.864	.0717	.0111	.0606	-----
	June 26, 1922	July 5	6.60	2.24	8.84	.979	.0940	.0184	.0756	9.74
	July 16, 1923	July 18	5.64	2.23	7.87	1.498	.0906	.0341	.0565	9.89
	July 23, 1924	Aug. 6	5.69	1.09	6.78	.636	.0895	.0365	.0530	-----
Lyman	Aug. 25, 1921	Aug. 30	9.48	1.18	10.66	.627	.2125	.1725	.0400	-----
	Aug. 19, 1922	Aug. 29	9.47	2.11	11.58	.685	.5229	.2105	.3124	15.88
	Sept. 13, 1924	Sept. 22	8.61	3.29	11.90	.511	.4340	.2260	.2080	-----
	Aug. 26, 1925	Sept. 3	9.98	2.48	12.46	.540	.5200	.2935	.2265	16.76
Magg	Sept. 25, 1922	Dec. 9	8.11	.91	9.02	.463	.0545	.0387	.0158	-----
	Oct. 4, 1923	Nov. 23	7.72	3.91	11.63	.520	.1275	.0635	.0640	12.60
	Oct. 4, 1924	Oct. 14	7.13	2.62	9.75	.647	.1390	.0549	.0841	-----
	Oct. 1, 1925	Oct. 13	8.03	1.65	9.68	.365	.0999	.0439	.0560	11.39
Magog	Sept. 28, 1922	Nov. 14	8.12	2.80	10.92	.426	.0858	.0168	.0690	13.11
	Sept. 23, 1923	Sept. 6	9.56	2.93	12.49	.663	.1115	.0360	.0755	14.80
	Sept. 27, 1924	Oct. 10	7.34	1.54	8.88	.627	.1870	.0865	.1005	-----
	Sept. 15, 1925	Sept. 25	8.02	1.00	9.02	.463	.0844	.0277	.0567	11.45
Mann	Oct. 13, 1922	Oct. 20	8.58	2.95	11.53	.558	.1088	.0495	.0593	13.71
	Oct. 20, 1923	Oct. 22	8.36	5.26	13.62	.690	.1112	.0477	.0635	16.31
	Nov. 4, 1924	Dec. 1	8.92	1.48	10.40	.580	.1090	.0169	.0921	-----
	Sept. 25, 1925	Nov. 16	10.44	2.98	13.42	.417	.1330	.0686	.0644	16.75
McMahon	Aug. 7, 1922	Aug. 8	6.92	.58	7.50	.983	.1349	.0287	.1062	10.07
	Aug. 15, 1923	Aug. 17	6.36	1.88	8.24	1.016	.0870	.0378	.0492	9.81
	Aug. 29, 1924	Sept. 3	6.35	1.35	7.70	.752	.0937	.0214	.0723	-----
	Aug. 1, 1925	Sept. 8	6.06	2.68	8.74	.508	.0876	.0328	.0548	10.94
Melon	Sept. 25, 1920	Oct. 6	8.98	1.45	10.43	.354	.1032	.0237	.0795	11.91
	Sept. 1, 1922	Sept. 14	7.25	3.23	10.48	.505	.1190	.0547	.0643	14.03
	Oct. 20, 1923	Oct. 26	7.70	1.74	9.44	.277	.0858	.0403	.0453	10.14
	Oct. 1, 1925	Oct. 12	9.14	1.10	10.24	.195	.1246	.0495	.0751	-----
Menagere	Sept. 30, 1922	Dec. 5	9.20	2.58	11.78	.463	.1084	.0622	.0462	14.51
	Oct. 4, 1923	Nov. 28	8.81	3.91	12.72	.398	.0981	.0459	.0522	15.34
	Oct. 4, 1924	Oct. 9	6.65	3.61	10.26	.397	.0745	.0282	.0463	-----
	Sept. 23, 1925	Nov. 3	6.38	1.43	7.81	.188	.0825	.0400	.0425	9.97
Missouri Pippin	Oct. 2, 1922	Oct. 5	7.60	1.70	9.30	.418	.1017	.0145	.0872	11.59
	Sept. 8, 1923	Oct. 18	6.62	4.15	10.77	.620	.0805	.0205	.0600	13.29
	Nov. 4, 1924	Nov. 25	6.97	4.93	11.90	.584	.0947	.0329	.0618	-----
	Oct. 5, 1925	Nov. 24	8.20	4.95	13.15	.490	.1390	.0490	.0900	16.83
Morris Red	Sept. 21, 1921	Oct. 18	10.02	2.44	12.46	.515	.0675	.0135	.0540	-----
	Oct. 3, 1922	Oct. 16	6.58	4.46	11.04	.279	.0763	.0132	.0631	15.30
	Sept. 8, 1923	do.	7.50	4.61	12.11	.457	.0960	.0428	.0532	15.74
	Oct. 8, 1924	Oct. 14	8.14	2.26	10.40	.247	.1167	.0542	.0625	-----
Nickajack	Oct. 10, 1922	Oct. 12	7.65	2.13	9.78	.451	.0877	.0316	.0561	13.81
	Oct. 12, 1923	Oct. 22	8.72	5.06	13.78	.330	.0935	.0361	.0574	15.99
	Nov. 4, 1924	Nov. 22	9.70	4.32	14.02	.377	.0915	.0342	.0573	-----
	Oct. 12, 1925	Nov. 23	10.30	1.46	11.76	.274	.1440	.0514	.0926	15.42

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 4 years— Continued. Newtown Spitz- enburg.	Oct. 3, 1922	Oct. 5	7.22	2.96	10.18	0.510	0.0771	0.0228	0.0543	-----
	Oct. 4, 1923	Nov. 19	7.44	3.87	11.31	.367	.0852	.0442	.0410	14.02
	Oct. 18, 1924	Nov. 25	6.31	1.61	7.92	.325	.0702	.0314	.0388	-----
	Sept. 25, 1925	Oct. 17	7.02	2.98	10.00	.273	.0760	.0302	.0458	11.88
Paragon-----	Sept. 2, 1921	Oct. 3	9.02	2.64	11.66	.507	-----	-----	-----	13.71
	Oct. 26, 1923	Nov. 5	8.10	3.79	11.89	.384	.1260	.0480	.0780	-----
	Nov. 4, 1924	Nov. 22	6.00	2.26	8.26	.332	.0955	.0355	.0600	-----
	Nov. 6, 1925	Nov. 25	10.58	2.30	12.88	.308	.1205	.0601	.0604	13.47
Pawpaw-----	Aug. 29, 1922	Sept. 16	7.41	3.55	10.96	.611	.1174	.0505	.0609	13.87
	Sept. 28, 1923	Nov. 24	8.50	4.27	12.77	.885	.1110	.0400	.0710	15.70
	Sept. 24, 1924	Oct. 3	5.90	3.24	9.14	.623	.0945	.0165	.0780	-----
	Oct. 1, 1925	Oct. 12	8.22	3.89	12.11	.482	.1170	.0453	.0717	14.67
Peck Pleasant....	Oct. 3, 1921	Nov. 4	10.10	2.07	12.17	.568	-----	-----	-----	-----
	Oct. 19, 1923	Oct. 29	7.38	6.46	13.84	.480	.0940	.0418	.0522	14.97
	Oct. 11, 1924	Oct. 21	9.16	2.10	11.26	.570	.1100	.0465	.0635	-----
	Oct. 7, 1925	Nov. 18	8.63	2.77	11.40	.292	.1125	.0405	.0720	13.02
Piper-----	Aug. 13, 1920	Aug. 26	8.24	2.66	10.90	.628	-----	-----	-----	-----
	June 27, 1922	June 30	8.85	.50	9.35	.564	.1614	.0408	.1206	10.67
	July 10, 1923	July 30	6.40	2.40	8.80	.580	.2180	.1010	.1170	10.46
	July 23, 1924	do-----	5.86	1.02	6.88	.353	.1370	.0545	.0825	-----
Rabum-----	Sept. 23, 1922	Nov. 16	8.81	1.77	10.58	.207	.0989	.0656	.0333	13.74
	Sept. 15, 1923	Dec. 5	8.88	2.80	11.68	.362	.1010	.0497	.0513	13.54
	Sept. 7, 1924	Sept. 20	6.85	2.09	8.94	.274	.0980	.0490	.0490	-----
	Sept. 23, 1925	Sept. 26	8.06	2.40	10.46	.365	.1011	.0443	.0568	13.84
Ramsdell Sweet.	Aug. 18, 1920	Sept. 11	9.62	2.00	11.62	.227	-----	-----	-----	-----
	Sept. 1, 1922	Sept. 5	9.03	2.15	11.18	.253	.1320	.0388	.0932	13.25
	Sept. 13, 1924	Sept. 24	8.08	2.78	10.86	.118	.1605	.0575	.1030	-----
	Oct. 7, 1925	Oct. 16	8.12	1.78	9.90	.078	.1460	.0475	.0985	12.10
Rome Beauty----	Oct. 7, 1922	Oct. 9	6.62	3.79	10.41	.360	.0877	.0284	.0613	11.99
	Oct. 9, 1923	Oct. 18	6.88	5.23	12.11	.392	.0720	.0164	.0556	13.41
	Nov. 4, 1924	Nov. 29	6.20	2.02	8.22	.264	.0836	.0146	.0690	11.62
	Sept. 23, 1925	Nov. 3	5.99	2.73	8.72	.273	.0993	.0329	.0664	11.28
Ronk-----	Sept. 18, 1922	Nov. 18	7.64	2.52	10.16	.524	.1187	.0256	.0931	12.96
	Oct. 8, 1923	Oct. 12	7.50	5.58	13.08	.720	.0925	.0315	.0610	14.81
	Oct. 11, 1924	Oct. 22	6.00	3.21	9.21	.566	.1085	.0455	.0640	-----
	Sept. 23, 1925	Oct. 23	5.60	2.67	8.27	.390	.0760	.0300	.0460	10.48
Roxbury Russet.	Oct. 4, 1920	Nov. 2	9.13	2.75	11.88	.557	-----	-----	-----	13.03
	Oct. 4, 1923	Nov. 5	6.29	5.98	12.27	.614	.1130	.0471	.0659	16.84
	Oct. 8, 1924	Oct. 22	6.16	3.94	10.10	.532	.0945	.0373	.0572	-----
	Oct. 1, 1925	Oct. 14	8.60	4.60	13.20	.502	.1200	.0365	.0595	16.41
Salome-----	Sept. 23, 1922	Dec. 9	8.80	1.60	10.40	.654	.0466	.0090	.0376	13.25
	Oct. 20, 1923	Oct. 23	8.41	4.79	13.20	.667	.0831	.0299	.0532	14.08
	Oct. 8, 1924	Oct. 10	7.58	2.98	10.56	.773	.0970	.0190	.0780	-----
	Sept. 15, 1925	do-----	10.06	2.15	12.21	.482	.0927	.0285	.0642	-----
Santa-----	Oct. 20, 1922	Oct. 30	7.12	3.04	10.16	.421	.1168	.0496	.0672	11.05
	Oct. 26, 1923	Nov. 2	7.36	2.97	10.33	.745	.0720	.0310	.0410	12.12
	Nov. 5, 1924	Dec. 2	7.48	1.37	8.82	.652	.0945	.0177	.0768	-----
	Oct. 16, 1925	Oct. 31	6.66	2.92	9.58	.628	.0722	.0232	.0492	12.18
Scott Winter-----	Sept. 23, 1922	Sept. 26	6.73	3.39	10.12	1.030	.0825	.0434	.0391	12.36
	Oct. 4, 1923	Nov. 5	6.56	3.62	10.18	.930	.1029	.0370	.0659	12.16
	Sept. 27, 1924	Oct. 4	10.26	6.30	16.56	.976	.1030	.0403	.0627	18.33
	Sept. 22, 1925	Oct. 16	7.22	2.25	9.47	.712	.1108	.0398	.0710	12.26
Shoemaker-----	July 16, 1922	July 19	6.44	1.78	8.22	.600	.1124	.0102	.1022	11.54
	July 20, 1923	July 23	6.06	2.58	8.64	.747	.1095	.0193	.0902	10.22
	July 29, 1924	Aug. 7	7.80	2.08	9.88	.374	.1535	.0598	.0937	-----
	July 21, 1925	Aug. 1	6.54	3.20	9.74	.383	.1140	.0540	.0600	-----

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							Total solids
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astrin-gency	Tan-nin	Non-tannin astrin-gency	
Fruiting 4 years— Continued. Smuth.....	Aug. 19, 1921	Sept. 5	8.64	2.93	11.57	0.453				
	Aug. 14, 1922	Aug. 16	9.98	2.98	12.96	.583	0.1267	0.0439	0.0828	13.36
	Aug. 29, 1923	Sept. 5	10.84	2.16	12.50	.475	.1200	.0595	.0605	14.60
	Aug. 29, 1924	Sept. 10	8.00	1.77	9.77	.337	.1017	.0587	.0430	13.29
Springdale.....	Oct. 28, 1920	Nov. 1	7.72	1.11	8.83	.660				11.20
	Oct. 20, 1922	Oct. 24	7.45	2.73	10.18	.454	.1274	.0557	.0717	13.84
	Oct. 20, 1923	Oct. 31	8.44	4.28	12.72	.555	.1535	.0395	.1140	13.98
	Nov. 4, 1924	Dec. 2	7.70	1.66	9.36	.325	.1290	.0487	.0803	-----
Stanard.....	Sept. 1, 1920	Sept. 11	8.09	1.20	9.29	.550				
	Aug. 19, 1922	Aug. 25	7.20	2.39	9.59	.553	.1011	.0214	.0797	11.80
	Sept. 3, 1923	Sept. 12	8.04	2.74	10.78	.426	.1330	.0550	.0780	11.83
	Sept. 15, 1924	Sept. 24	6.00	2.92	8.92	.400	.1210	.0564	.0646	-----
Striped Pippin....	Oct. 4, 1920	Nov. 2	7.46	2.93	10.39	.680				11.76
	Sept. 23, 1922	Nov. 1	6.14	3.09	9.23	.710	.1478	.0673	.0805	10.81
	Sept. 3, 1923	Oct. 8	7.58	3.20	10.78	.468	.0918	.0395	.0523	13.66
	Oct. 1, 1925	Oct. 14	6.94	2.07	9.01	.371	.1185	.0521	.0664	12.34
Stuart.....	Sept. 13, 1921	Oct. 6	6.31	3.64	9.95	.711				
	Oct. 13, 1922	Oct. 14	5.78	3.98	9.76	.545	.0842	.0325	.0517	11.25
	Oct. 8, 1923	Nov. 17	5.80	4.10	9.70	.356	.0727	.0213	.0514	11.36
	Sept. 25, 1925	Oct. 20	7.70	3.84	11.54	.357	.0868	.0284	.0584	13.45
Sweet Romanite..	Oct. 11, 1921	Nov. 3	9.98	4.13	14.11	.303				
	Oct. 10, 1922	Oct. 17	8.25	6.28	14.53	.198	.1070	.0421	.0649	16.55
	Oct. 12, 1923	Oct. 19	8.00	5.14	13.14	.342	.1140	.0202	.0938	14.26
	Oct. 18, 1924	Nov. 1	7.70	5.40	13.10	.163	.1130	.0260	.0870	-----
Terry.....	Sept. 2, 1921	Sept. 24	8.16	2.75	10.91	.662				
	Oct. 22, 1922	Oct. 24	5.98	4.55	10.53	.786	.1133	.0275	.0858	13.66
	Oct. 26, 1923	Oct. 31	5.90	6.24	12.14	.746	.1232	.0460	.0772	16.40
	Nov. 4, 1924	Nov. 15	5.72	6.39	12.11	.667	.1217	.0422	.0795	-----
Twisty.....	Aug. 6, 1921	Aug. 30	8.78	.70	9.48	.500				
	Aug. 11, 1922	Aug. 14	10.02	.19	10.21	1.023	.0848	.0092	.0756	12.68
	Aug. 23, 1923	Aug. 30	7.73	1.08	8.81	.487	.0606	.0203	.0403	10.97
	Sept. 1, 1924	Sept. 4	6.22	1.04	7.26	.332	.0692	.0175	.0417	10.18
Victoria Sweet...	Aug. 19, 1922	Aug. 21	7.14	4.20	11.34	.444	.0909	.0266	.0643	13.33
	Aug. 23, 1923	Sept. 5	7.93	2.84	10.77	.249	.1282	.0571	.0711	12.78
	Sept. 13, 1924	Sept. 18	7.96	3.38	11.34	.178	.1190	.0457	.0733	-----
	Sept. 5, 1925	Sept. 14	8.10	2.98	11.08	.124	.1210	.0576	.0634	13.76
Wallace Howard..	Sept. 23, 1922	Nov. 8	7.20	3.38	10.58	.330	.1371	.0984	.0407	-----
	Sept. 28, 1923	Nov. 30	7.06	3.25	10.31	.326	.0982	.0460	.0522	13.04
	Sept. 15, 1924	Oct. 3	5.90	3.24	9.14	.410	.0980	.0457	.0523	-----
	Sept. 16, 1925	Oct. 8	7.96	3.42	11.38	.292	.1070	.0504	.0566	13.42
Weaver.....	Sept. 23, 1922	Nov. 17	6.52	1.86	8.38	.154	.0908	.0271	.0637	11.54
	Sept. 8, 1923	Sept. 24	7.64	4.05	11.69	.221	.1150	.0570	.0580	13.00
	Oct. 1, 1924	Oct. 6	6.72	3.20	9.92	.208	.1090	.0360	.0730	-----
	Sept. 23, 1925	Sept. 24	7.76	2.59	10.35	.156	.1127	.0495	.0632	13.07
Wells.....	Sept. 28, 1922	Nov. 14	6.69	3.15	9.84	.406	.0770	.0133	.0637	12.19
	Sept. 13, 1923	Nov. 28	6.83	3.06	9.89	.376	.0779	.0293	.0486	11.02
	Sept. 13, 1924	Sept. 18	5.35	3.04	8.39	.621	.0567	.0267	.0300	-----
	Oct. 1, 1925	Oct. 14	6.40	2.12	8.52	.417	.0710	.0168	.0542	10.30
Willow.....	Sept. 24, 1921	Oct. 16	9.02	2.32	11.34	.444				
	Sept. 25, 1922	Sept. 30	5.84	2.98	8.82	.682	.0799	.0304	.0495	11.58
	Oct. 12, 1923	Oct. 20	6.02	5.38	11.40	.587	.0960	.0249	.0711	12.19
	Oct. 4, 1924	Oct. 30	6.00	4.28	10.28	.577	.1080	.0357	.0723	-----
Wingate.....	Aug. 15, 1922	Aug. 17	7.39	1.15	8.54	.794	.1390	.0389	.1001	10.94
	Aug. 29, 1923	Sept. 11	5.86	2.03	7.89	.462	.1010	.0518	.0492	9.63
	Aug. 29, 1924	Sept. 4	7.14	2.78	9.92	.503	.1222	.0454	.0768	-----
	Sept. 1, 1925	do.....	5.72	2.34	8.06	.424	.0920	.0355	.0565	11.18

See footnote at end of table.

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 4 years— Continued. Winter Banana...	Aug. 23, 1921	Sept. 19	7.40	3.45	10.85	0.293	-----	-----	-----	-----
	Sept. 6, 1922	Sept. 13	6.63	4.10	10.73	.317	0.1181	0.0634	0.0547	12.42
	Sept. 5, 1923	do-----	8.20	2.33	10.53	.371	.1530	.0540	.0990	12.02
	Sept. 13, 1925	Oct. 5	9.16	1.68	10.84	.182	.1390	.0570	.0820	13.18
Winter Paradise...	Oct. 16, 1922	Oct. 18	8.76	1.20	9.96	.114	.1447	.0860	.0587	12.04
	Oct. 19, 1923	Oct. 25	8.84	3.34	12.18	.132	.1435	.0759	.0676	14.15
	Oct. 28, 1924	Nov. 1	7.02	2.40	9.42	.107	.1667	.0595	.1072	-----
	Oct. 10, 1925	Oct. 31	9.32	2.16	11.48	.092	.1620	.0670	.0950	13.84
Yellow Trans- parent.	June 26, 1922	June 30	6.34	1.56	7.90	.680	.1942	.1125	.0817	9.24
	Sept. 5, 1923	July 18	7.35	2.07	9.42	1.400	.2270	.0876	.1394	12.17
	July 23, 1924	July 30	5.31	.77	6.08	.439	.1360	.0380	.0980	9.69
	July 11, 1925	July 17	5.92	1.61	7.53	.486	.0753	.0255	.0498	-----
Yopp-----	Oct. 19, 1922	Oct. 20	7.62	2.91	10.53	.329	.0947	.0345	.0602	11.92
	Sept. 28, 1923	Oct. 2	6.86	3.65	10.51	.314	.0813	.0221	.0592	12.32
	Sept. 24, 1924	Oct. 7	6.20	2.76	8.96	.374	.1280	.0655	.0625	-----
	Sept. 25, 1925	Sept. 27	7.12	1.75	8.87	.274	.0920	.0429	.0491	11.23
York Imperial...	Sept. 13, 1921	Oct. 16	9.00	2.04	11.04	.588	-----	-----	-----	-----
	Sept. 25, 1922	Oct. 12	10.71	1.58	12.29	.491	.0657	.0050	.0607	13.49
	Nov. 1, 1923	Nov. 2	8.36	4.12	12.48	.467	.0770	.0315	.0455	14.80
	Oct. 14, 1924	Oct. 17	7.13	2.43	9.56	.463	.0516	.0136	.0380	-----
Fruiting 3 years: All-Summer-----	July 27, 1920	Aug. 3	7.30	1.04	8.34	.631	-----	-----	-----	10.00
	July 20, 1922	July 21	6.07	1.97	8.04	.466	.1287	.0296	.0991	-----
	Aug. 2, 1923	Aug. 6	7.34	4.05	11.39	.895	.1410	.0840	.0570	12.39
Alton-----	Oct. 10, 1922	Oct. 12	6.44	4.00	10.44	.634	.0771	.0140	.0631	12.54
	Oct. 12, 1923	Oct. 25	6.06	2.72	8.80	.374	.0472	.0180	.0292	9.48
	Oct. 4, 1924	Oct. 17	7.31	1.24	8.55	.570	.0487	.0127	.0360	-----
Arkansas Beauty...	Oct. 26, 1923	Nov. 2	7.64	4.12	11.76	.460	.0944	.0482	.0462	15.20
	Oct. 8, 1924	Oct. 16	7.00	2.88	9.88	.515	.0865	.0325	.0540	-----
	Oct. 14, 1925	Nov. 7	7.04	2.86	9.90	.338	.0980	.0522	.0458	11.91
Baltimore-----	Sept. 23, 1922	Nov. 1	7.66	2.58	10.24	.241	.1212	.0336	.0876	-----
	Oct. 12, 1923	Oct. 18	8.20	4.70	12.90	.352	.0900	.0283	.0617	14.49
	Oct. 18, 1924	Oct. 23	6.12	2.18	8.30	.242	.0952	.0344	.0608	-----
Barnes-----	Sept. 20, 1921	Oct. 19	8.64	4.13	12.77	.456	-----	-----	-----	-----
	Oct. 20, 1924	Nov. 8	8.88	3.02	11.90	.290	.1130	.0480	.0650	-----
	Oct. 7, 1925	Nov. 21	9.32	2.60	11.92	.218	.1152	.0320	.0832	14.10
Baxter-----	Sept. 13, 1923	Sept. 27	6.94	3.87	10.81	.634	.1010	.0308	.0702	13.06
	Sept. 13, 1924	Oct. 7	4.80	2.24	7.04	.340	.0780	.0257	.0523	-----
	Sept. 17, 1925	Sept. 24	7.02	2.30	9.32	.352	.0932	.0414	.0518	11.81
Bismarck-----	Aug. 12, 1921	Sept. 6	8.16	3.13	11.29	.427	-----	-----	-----	-----
	Sept. 28, 1922	Dec. 14	7.86	2.02	9.88	.688	.0826	.0227	.0599	10.57
	Oct. 8, 1924	Oct. 15	8.63	.41	9.04	.630	.1435	.0550	.0885	11.51
Blenheim-----	Sept. 13, 1922	Dec. 12	7.05	4.65	11.70	.646	.0650	.0237	.0413	13.02
	Sept. 13, 1923	Nov. 21	7.10	7.01	14.11	.870	.1455	.0721	.0734	16.00
	Oct. 8, 1924	Oct. 21	6.64	4.58	11.22	.590	.0945	.0423	.0522	-----
Brown (Notting- ham Brown.)	Sept. 23, 1922	Nov. 18	8.30	2.86	11.16	.515	.1056	.0194	.0860	14.50
	Oct. 8, 1923	Oct. 17	7.67	3.87	11.54	.482	.0916	.0289	.0617	14.56
	Oct. 4, 1924	Oct. 20	7.76	3.00	10.76	.590	.1010	.0470	.0540	-----
Buckingham-----	Sept. 23, 1922	Dec. 9	7.65	2.05	9.70	.431	.0448	.0343	.0105	12.19
	Sept. 27, 1923	Oct. 8	6.26	3.39	9.65	.456	.0994	.0463	.0531	11.67
	Oct. 14, 1924	Oct. 17	6.00	1.42	7.42	.356	.0780	.0335	.0445	-----
Colton-----	July 15, 1920	July 28	8.54	.42	8.96	.641	-----	-----	-----	-----
	June 10, 1922	June 30	7.54	.70	8.24	.713	.2095	.0716	.1379	10.31
	July 23, 1924	July 30	4.65	1.57	6.22	.429	.0970	.0340	.0630	-----
Colvert-----	Aug. 11, 1922	Aug. 14	8.46	2.39	10.85	.769	.1553	.0552	.1001	14.63
	Sept. 16, 1923	Nov. 22	6.08	3.16	9.24	.425	.1455	.0685	.0770	11.16
	Sept. 15, 1924	Sept. 19	6.49	2.83	9.32	.494	.1082	.0392	.0690	-----

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astrin-gency	Tan-nin	Non-tannin astrin-gency	Total Solids
Fruiting 3 years—Continued.										
Cotter.....	Sept. 23, 1922	Dec. 14	8.36	1.88	10.24	0.532	0.0668	0.0185	0.0483	12.52
	Oct. 9, 1923	Oct. 18	7.58	3.08	10.66	.466	.0617	.0172	.0445	12.51
	Oct. 4, 1924	Oct. 6	7.02	2.91	9.93	.458	.0935	.0412	.0523	-----
Covert.....	Sept. 28, 1922	Nov. 16	9.66	1.20	10.86	.244	.0832	.0368	.0464	13.23
	Sept. 9, 1923	Oct. 18	9.26	2.24	11.50	.316	.1130	.0495	.0635	13.68
	Sept. 16, 1924	Oct. 8	8.26	1.10	9.36	.349	.0978	.0236	.0742	-----
Cox No. 11.....	Aug. 14, 1922	Aug. 17	7.00	3.58	11.18	.708	.1093	.0450	.0643	12.30
	Aug. 17, 1923	do	6.48	2.41	8.89	.922	.0728	.0303	.0425	10.19
	Aug. 18, 1924	Aug. 25	5.76	2.58	8.34	.706	.0417	.0157	.0260	-----
Cross.....	Aug. 12, 1922	Aug. 15	7.82	1.20	9.02	.515	.1482	.0665	.0817	12.55
	Sept. 8, 1923	Sept. 19	8.26	2.57	10.83	.346	.1485	.0645	.0840	13.35
	Aug. 29, 1924	Sept. 10	7.49	2.04	9.53	.439	.1353	.0798	.0555	13.16
Daniel.....	Aug. 7, 1922	Aug. 8	8.31	3.78	12.09	.632	.1338	.0316	.1022	13.09
	Aug. 20, 1923	Aug. 24	7.47	3.20	10.67	.452	.0985	.0353	.0632	11.97
	Aug. 29, 1924	Sept. 6	8.04	3.02	11.06	.311	.0847	.0305	.0542	-----
Early Richmond.	July 16, 1922	July 18	5.39	3.25	8.64	.861	.0868	.0122	.0746	9.69
	July 14, 1923	do	5.95	2.79	8.74	1.334	.1026	.0256	.0770	-----
	July 28, 1924	Aug. 4	5.24	2.37	7.61	.967	.0855	.0222	.0633	-----
Family.....	Aug. 23, 1922	Sept. 5	7.90	.22	8.12	.837	.0776	.0172	.0604	9.86
	Sept. 13, 1923	Sept. 27	6.00	5.09	11.09	.800	.0835	.0205	.0630	-----
	Sept. 23, 1924	Oct. 2	3.76	3.10	6.86	.505	.0515	.0075	.0440	-----
Gano.....	Sept. 10, 1921	Oct. 11	6.25	2.93	9.18	.510	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 10	7.44	2.64	10.08	.406	.1043	.0324	.0719	11.73
	Sept. 28, 1923	Dec. 3	7.38	3.53	10.91	.321	.1094	.0500	.0594	11.62
Gideon.....	Aug. 18, 1920	Sept. 16	8.94	1.08	10.02	.673	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 6	7.62	2.55	10.17	.456	.0956	.0477	.0479	12.31
	Sept. 5, 1924	Sept. 19	7.46	1.02	8.48	.362	.1080	.0605	.0475	-----
Golden Pippin....	Aug. 8, 1922	Aug. 10	6.80	2.02	8.82	.658	.0991	.0184	.0907	11.65
	Aug. 13, 1923	Aug. 16	5.89	4.69	10.58	.632	.1020	.0406	.0614	10.79
	Aug. 20, 1924	Sept. 3	6.11	1.89	8.00	.313	.0904	.0181	.0723	-----
Golden Sweet....	July 31, 1920	Aug. 6	8.86	1.12	9.98	.144	.1280	.0460	.0820	11.40
	July 25, 1922	July 26	8.37	1.20	9.57	.276	.1768	.0521	.1247	-----
	Aug. 2, 1923	Aug. 6	6.09	3.43	9.52	.200	.1880	.0845	.1035	10.72
Grosh.....	Sept. 6, 1922	Dec. 11	9.12	2.54	11.66	.341	.0527	.0132	.0305	14.14
	Sept. 16, 1923	Nov. 12	6.68	5.64	12.32	.488	.1233	.0668	.0565	16.54
	Sept. 13, 1924	Sept. 25	5.58	2.52	8.10	.405	.0830	.0258	.0572	-----
Heidemeyer.....	Sept. 6, 1922	Sept. 15	9.22	1.60	10.82	.557	.0975	.0406	.0560	12.85
	Aug. 20, 1923	Sept. 5	7.02	4.18	11.20	.488	.1175	.0465	.0710	14.08
	Sept. 7, 1924	Sept. 16	6.36	3.94	10.30	.494	.1190	.0775	.0415	-----
Henry Clay.....	July 6, 1922	July 7	6.54	1.08	7.62	.972	.1471	.0521	.0950	-----
	July 6, 1923	July 13	5.92	2.96	8.88	.595	.1221	.0591	.0630	10.49
	Aug. 4, 1924	Aug. 4	5.24	1.36	6.60	.373	.1050	.0285	.0765	-----
Hogg.....	Aug. 3, 1922	do	6.80	2.71	9.51	1.454	.2023	.0828	.1195	13.14
	Aug. 14, 1923	Aug. 21	8.30	5.44	13.74	2.259	.1560	.0743	.0817	14.99
	Sept. 2, 1924	Sept. 15	6.11	3.93	10.04	1.066	.1262	.0690	.0572	-----
Indiana Favorite.	Sept. 23, 1922	Nov. 16	7.55	1.99	9.54	.344	.0788	.0455	.0333	13.73
	Oct. 12, 1923	Oct. 30	7.44	4.99	12.43	.318	.0745	.0283	.0462	13.71
	Oct. 18, 1924	do	7.84	4.94	12.78	.388	.0690	.0210	.0480	-----
Irish Peach.....	July 22, 1920	July 30	6.03	2.00	8.03	.520	-----	-----	-----	-----
	July 14, 1922	July 17	5.80	1.93	7.73	.581	.1390	.0511	.0879	11.10
	July 10, 1923	July 18	5.85	1.30	7.15	.552	.2750	.1210	.1540	-----
Julian de Paul-mier.	Sept. 3, 1923	Sept. 19	9.52	3.38	12.90	.350	.6100	.3200	.2900	15.61
	Sept. 7, 1924	Sept. 18	8.35	3.37	11.72	.185	.4720	.1580	.3140	-----
	Oct. 5, 1925	Oct. 12	8.80	3.52	12.32	.182	.3260	.0860	.2400	15.09



TABLE 1.—*Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued*

Variety	Date picked	Date of analysis	Constituents (per cent)								
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after in- ver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids	
Fruiting 3 years— Continued.											
Kay-----	Aug. 18, 1921 Sept. 5, 1923 Sept. 15, 1924	Sept. 16 Sept. 14 Oct. 2	6.04 6.56 7.10	1.43 2.65 2.04	7.47 9.21 9.14	0.582 .562 .445	0.1113 .1160 .1335	0.0136 .0520 .0695	0.0977 .0640 .0640	----- 9.60 -----	
King David-----	Aug. 30, 1921 Sept. 23, 1922 Sept. 19, 1923	Nov. 1 Nov. 17 Nov. 16	7.79 7.79 9.28	2.91 4.52 4.42	10.70 12.31 13.70	.372 .561 .651	.0749 .0692	.0263 .0092	.0486 .0600	15.56 14.48	
Melo Gelato-----	Oct. 13, 1922 Oct. 12, 1923 Oct. 28, 1924	Oct. 16 Oct. 26 Nov. 15	8.27 8.69 7.66	2.08 3.07 1.82	10.35 11.76 9.48	.279 .238 .206	.0833 .1430 .0937	.0377 .0726 .0439	.0456 .0704 .0498	12.30 15.08 -----	
Milam-----	Sept. 25, 1920 Aug. 30, 1921 Sept. 23, 1923	Oct. 2 Sept. 16 Oct. 2	8.00 7.92 6.72	.56 4.18 3.66	8.56 12.10 10.38	.568 .668 .650	.0795 .0820 .1000	.0218 .0144 .0377	.0577 .0676 .0623	11.02 ----- 12.57	
Munson-----	Aug. 14, 1920 Aug. 4, 1922 Aug. 16, 1923	Aug. 17 Aug. 7 Aug. 24	7.46 8.21 7.22	1.75 3.07 4.47	9.21 10.69 11.69	.230 .254 .246	.0930 .1768 .1050	.0180 .0317 .0400	.0750 .1451 .0650	----- 11.29 13.30	
Munster-----	Aug. 15, 1922 Aug. 13, 1923 Sept. 5, 1924	Aug. 16 Aug. 21 Sept. 15	8.92 8.13 7.30	2.10 3.04 3.03	11.02 11.17 10.33	.365 .390 .217	.1246 .1310 .1452	.0388 .0685 .1025	.0858 .0625 .0427	12.86 13.09 -----	
Northern Spy-----	Sept. 28, 1922 Aug. 29, 1924 Sept. 16, 1925	Nov. 3 Oct. 8 Sept. 22	8.12 6.54 8.06	2.86 3.16 3.32	10.98 9.70 11.38	.393 .567 .495	.1239 .1055 .1340	.0425 .0403 .0656	.0814 .0652 .0684	13.09 ----- 13.61	
Oakland-----	Sept. 6, 1922 Sept. 19, 1923 Sept. 13, 1924	Sept. 20 Nov. 14 Sept. 24	8.00 8.00 6.46	1.59 4.26 1.80	9.59 12.26 8.26	.233 .294 .279	.0738 .1045 .0604	.0243 .0464 .0149	.0495 .0581 .0455	12.34 15.04 -----	
Ohio Nonpareil-----	Aug. 23, 1922 Sept. 15, 1924 Aug. 28, 1925	Aug. 28 Sept. 22 Sept. 24	7.88 6.20 5.28	2.74 3.22 2.55	10.62 9.42 7.83	.522 .265 .163	.1104 .0880 .0725	.0431 .0348 .0325	.0673 .0532 .0400	14.29 ----- 9.27	
Ortley-----	Sept. 23, 1922 Sept. 27, 1923 Oct. 8, 1924	Sept. 23 Sept. 29 Oct. 21	6.04 8.00 7.64	3.23 3.24 2.29	9.27 11.24 9.93	.522 .732 .400	.0634 .0842 .0713	.0348 .0342 .0318	.0286 .0500 .0395	11.42 13.24 -----	
Ozone-----	Sept. 28, 1922 Sept. 27, 1923 Oct. 1, 1924	Nov. 4 Oct. 10 Oct. 9	8.07 8.92 7.28	2.51 4.39 2.64	10.58 13.31 9.92	.342 .558 .540	.1248 .1003 .0960	.0319 .0206 .0275	.0929 .0797 .0685	12.93 16.29 -----	
Patten-----	Aug. 24, 1920 Aug. 15, 1922 Sept. 24, 1924	Sept. 17 Aug. 18 Oct. 2	6.40 7.55 5.38	.92 1.47 1.58	7.32 9.02 6.96	.678 .964 .630	.0513 .1226 .1250	.0113 .0347 .0578	.0400 .0879 .0672	9.64 ----- -----	
Pinnacle-----	Sept. 2, 1921 Sept. 13, 1922 Oct. 7, 1925	Sept. 24 Oct. 7 Nov. 21	8.08 7.33 6.76	3.40 2.28 3.08	11.48 9.61 9.84	.863 .647 .430	.0878 .1131 .1270	.0110 .0517 .0385	.0768 .0614 .0885	----- 12.32 11.40	
Précoce de Tunis.	July 21, 1920 July 13, 1922 July 16, 1923	July 25 July 18 July 26	6.80 6.24 7.76	1.54 1.89 3.87	8.34 8.13 11.63	.190 .348 .358	.2239 .1635 .3910	.0714 .0716 .1900	.1525 .0919 .2010	----- 9.51 13.92	
Primate-----	July 20, 1922 July 20, 1923 Aug. 6, 1924	July 21 July 23 Aug. 11	6.22 7.34 7.26	3.52 2.58 .38	9.74 9.92 7.64	.478 .890 1.385	.1287 .1424 .0740	.0310 .0887 .0318	.0977 .0537 .0422	10.94 11.32 -----	
Rambo-----	Sept. 2, 1920 Oct. 20, 1923 Sept. 14, 1924	Sept. 23 Oct. 26 Sept. 19	8.90 9.48 6.76	2.01 4.06 1.70	10.91 13.54 8.46	.388 .402 .398	.0920 .1002 .0795	.0352 .0445 .0070	.0568 .0557 .0725	12.88 14.84 -----	
Rebel-----	Sept. 23, 1922 Sept. 27, 1923 Sept. 26, 1924	Oct. 13 -----do----- Oct. 3	7.36 7.38 6.16	3.22 4.44 3.52	10.58 11.82 9.68	.253 .341 .325	.0841 .0615 .0753	.0275 .0201 .0254	.0566 .0414 .0499	13.31 13.58 -----	
Red June-----	July 15, 1922 July 20, 1923 July 28, 1924	July 17 July 23 Aug. 13	6.01 6.06 6.54	1.84 2.93 2.43	7.85 8.99 8.97	.551 .886 .350	.1584 .1373 .1180	.0603 .0713 .0390	.0981 .0660 .0790	10.56 10.30 -----	

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920–1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							Total solids
			Reducing sugar	Sucrose as invert sugar	Total sugar after inversion	Acid as malic	Total astrin-gency	Tannin	Non-tannin astrin-gency	
Fruiting 3 years—Continued. Red Keeper.....	Oct. 26, 1922	Oct. 29	8.32	2.80	11.12	0.220	0.1292	0.0531	0.0761	13.55
	Nov. 4, 1924	Dec. 2	7.88	2.62	10.50	.296	.1104	.0242	.0862	-----
	Oct. 12, 1925	Nov. 25	5.88	3.21	9.09	.208	.0800	.0300	.0500	9.83
Red Siberian.....	Aug. 1, 1922	Aug. 7	9.24	4.06	13.30	1.351	.5283	.2340	.2943	16.36
	Sept. 3, 1923	Sept. 18	8.18	4.80	12.98	.938	.6600	.4320	.2280	16.22
	Sept. 10, 1924	Sept. 16	7.49	2.13	9.62	.605	.5450	.3985	.1465	-----
Reynard.....	Sept. 1, 1922	Sept. 5	8.96	.98	9.94	.685	.0794	.0346	.0448	12.45
	Sept. 3, 1923	Sept. 11	7.96	1.13	9.09	.518	.0905	.0194	.0711	11.63
	Aug. 9, 1924	Sept. 4	7.99	2.47	10.46	.480	.0880	.0202	.0678	-----
Ruby Gem.....	July 25, 1922	July 26	6.44	1.95	8.39	.769	.1635	.0399	.1236	10.72
	Aug. 1, 1923	Sept. 12	6.00	4.94	10.94	.676	.1680	.0643	.1037	12.73
	Aug. 11, 1924	Aug. 25	6.66	1.84	8.50	.400	.1172	.0584	.0588	-----
Rymer.....	Aug. 28, 1920	Sept. 28	7.18	.80	7.98	.698	.0752	.0210	.0542	12.18
	Sept. 23, 1923	Dec. 8	6.66	2.44	9.10	.522	.0836	.0199	.0637	10.78
	Oct. 4, 1924	Oct. 11	6.74	2.34	9.08	.573	.1235	.0567	.0668	-----
Schroder.....	Nov. 1, 1920	Nov. 8	6.36	.68	7.04	.705	.0665	.0219	.0446	-----
	Oct. 4, 1923	do.....	8.52	2.58	11.10	.835	.1170	.0528	.0642	14.62
	Oct. 28, 1924	Nov. 25	6.82	2.20	9.02	.427	.0878	.0363	.0515	11.86
Sierra Beauty....	Sept. 23, 1922	Dec. 13	6.92	5.02	11.94	.837	.0677	.0184	.0493	14.22
	Oct. 12, 1923	Oct. 23	6.58	4.78	11.36	.602	.0695	.0165	.0530	12.83
	Oct. 21, 1924	do.....	7.02	2.28	9.30	.657	.0715	.0185	.0530	-----
Spencer Seedling..	Oct. 5, 1922	Oct. 13	9.97	2.81	12.78	.360	.1105	.0369	.0736	15.16
	Oct. 12, 1923	Oct. 23	8.30	5.76	14.06	.303	.1480	.0690	.0790	14.55
	Oct. 18, 1924	Oct. 30	8.54	2.98	11.52	.265	.1080	.0255	.0825	-----
Spice.....	Aug. 14, 1920	Aug. 16	8.20	1.68	9.88	.810	.0734	.0129	.0605	-----
	Aug. 11, 1922	Aug. 13	7.65	2.51	10.16	.870	.1022	.0174	.0848	11.38
	Aug. 21, 1923	Aug. 27	6.62	4.90	11.52	.775	.1135	.0450	.0685	13.64
Stark.....	Oct. 15, 1922	Oct. 18	7.39	3.30	10.69	.416	.0649	.0412	.0237	12.67
	Oct. 26, 1923	Nov. 2	8.97	4.89	13.86	.481	.0984	.0368	.0616	15.58
	Oct. 21, 1924	Dec. 1	7.72	1.64	9.36	.291	.0885	.0345	.0540	11.29
Starkey.....	Aug. 30, 1922	Sept. 5	9.26	2.28	11.54	.606	.1113	.0052	.1061	14.66
	Sept. 15, 1923	Nov. 17	7.47	3.30	10.77	.495	.1030	.0508	.0522	13.78
	Sept. 5, 1924	Sept. 16	7.49	3.43	10.92	.434	.0915	.0393	.0522	-----
Stinson.....	Aug. 14, 1922	Aug. 16	8.82	1.68	10.50	.674	.0950	.0082	.0868	13.04
	Aug. 15, 1923	Aug. 17	7.88	2.50	10.38	.640	.0940	.0352	.0588	12.10
	Aug. 29, 1924	Sept. 3	7.60	3.30	10.90	.366	.1105	.0417	.0688	-----
Summer Pound....	July 28, 1922	July 31	6.29	2.27	8.56	.733	.1001	.0225	.0776	10.96
	July 18, 1924	Aug. 25	5.88	3.00	8.88	.488	.0622	.0352	.0270	11.34
	Sept. 1, 1925	Sept. 4	5.14	2.49	7.63	.260	.0985	.0375	.0610	10.74
Townsend.....	July 28, 1922	July 31	6.28	2.95	9.21	.740	.1134	.0235	.0899	11.61
	Aug. 8, 1923	Aug. 13	7.06	3.37	10.43	.462	.0855	.0342	.0513	11.66
	Aug. 29, 1924	Sept. 6	8.14	2.96	11.10	.282	.0915	.0330	.0585	-----
Trader.....	July 28, 1922	July 31	6.87	2.01	8.88	.717	.1512	.0225	.1287	11.16
	Aug. 16, 1923	Aug. 24	8.34	4.92	13.26	.520	.2030	.1342	.0688	15.07
	Sept. 8, 1924	Sept. 8	7.38	2.96	10.34	.401	.1275	.0959	.0316	-----
Trumbull.....	Aug. 3, 1922	Aug. 7	8.34	1.85	10.19	.199	.1727	.0593	.1134	11.79
	Aug. 29, 1923	Sept. 5	9.18	3.75	12.93	.221	.1700	.0935	.0765	14.34
	Aug. 29, 1924	Sept. 12	6.86	3.02	9.88	.133	.1251	.0564	.0687	11.34
Utter.....	Aug. 23, 1922	Aug. 25	6.60	1.27	7.87	.482	.0848	.0174	.0674	10.77
	Sept. 5, 1923	Sept. 7	6.78	4.30	11.08	.534	.0975	.0275	.0700	11.19
	Sept. 5, 1924	Sept. 16	7.02	1.92	8.94	.319	.1150	.0610	.0540	-----
Wealthy.....	Aug. 11, 1922	Aug. 14	7.20	1.37	8.57	.709	.0848	.0061	.0787	10.37
	Aug. 23, 1923	Sept. 4	7.33	2.87	10.20	.622	.0796	.0203	.0593	11.79
	Aug. 29, 1924	Sept. 3	7.38	1.75	9.13	.665	.1140	.0575	.0565	11.20

TABLE 1.—Analytical results for the juices of 216 varieties of apples analyzed in three to six of the years 1920-1925—Continued

Variety	Date picked	Date of analysis	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar after inver- sion	Acid as malic	Total astrin- gency	Tan- nin	Non- tannin astrin- gency	Total solids
Fruiting 3 years— Continued White Pearmain.	Oct. 19, 1922	Oct. 19	7.92	5.14	13.06	0.583	0.0973	0.0309	0.0664	15.65
	Oct. 26, 1923	Oct. 31	7.22	6.64	13.86	.590	.0968	.0368	.0600	15.93
	Oct. 4, 1924	Oct. 21	7.64	3.47	11.11	.575	.1020	.0380	.0640	-----
Williams.....	July 25, 1923	July 30	6.67	4.10	10.77	.551	.1420	.0695	.0725	12.32
	July 29, 1924	Aug. 6	6.67	2.15	8.82	.296	.1275	.0606	.0669	-----
	July 21, 1925	July 25	6.71	1.53	8.24	.387	.0856	.0311	.0545	-----
Wilson June.----	Aug. 7, 1922	Aug. 9	7.51	3.42	10.93	.713	.1451	.0389	.1062	11.95
	Aug. 23, 1923	Aug. 30	7.40	2.52	9.92	.470	.1200	.0594	.0606	10.90
	Aug. 29, 1924	Sept. 2	6.26	3.03	9.29	.385	.0858	.0383	.0475	-----
Winter Smoke- house. <sup>1</sup>	Sept. 8, 1922	Nov. 4	8.15	3.37	11.52	.365	.1141	.0185	.0956	12.87
	Oct. 12, 1923	Nov. 14	7.65	4.13	11.78	.557	.1167	.0561	.0606	-----
	Sept. 24, 1924	Oct. 8	7.33	1.92	9.25	.496	.1128	.0516	.0612	-----
Wolseley.....	Oct. 13, 1922	Oct. 17	6.84	2.73	9.57	.697	.1000	.0430	.0570	12.90
	Oct. 12, 1923	Oct. 23	7.44	3.76	11.20	.730	.0945	.0295	.0650	12.74
	Oct. 18, 1924	Oct. 23	6.02	1.71	7.73	.692	.0675	.0246	.0429	-----
Yellow Siberian..	Aug. 1, 1922	Aug. 7	10.88	1.66	12.54	1.154	.4374	.1952	.2422	15.40
	Sept. 6, 1923	Sept. 18	6.67	4.53	11.20	1.010	.3450	.1982	.1468	13.02
	Aug. 29, 1924	Sept. 19	7.82	3.20	11.02	.593	.3760	.2335	.1425	-----
Zoar.-----	Aug. 2, 1921	Aug. 24	7.02	1.28	8.30	.722	-----	-----	-----	-----
	Aug. 7, 1922	Aug. 8	8.18	1.62	9.80	.975	.1471	.0368	.1103	11.02
	Aug. 21, 1923	Aug. 27	7.64	1.99	9.63	.454	.1150	.0598	.0552	10.64

\* The material employed in these analyses was taken from two trees originally planted as ornamentals in the grounds of the Arlington farm. The seeds were taken by D. N. Shoemaker, of the Office of Horticulture, in 1909 or 1910 from a single tree which is still standing in a piece of waste land adjacent to the farm. The very exceptional characters of the fruit attracted attention to it and led to the making of annual analyses upon the crop of the younger trees and an occasional analysis of that of the parent tree. These agree in all essential respects. The trees have been identified as *Pyrus angustifolia* by members of the Office of Horticulture.

† Two apples are known under this name. That here employed does not conform to the description given by Beach (?) but agrees closely in all respects with the description by Bunyard (g) and is believed to be the apple known in England under this name.

‡ The apple designated as Hort. No. 3050 was received as Jackson. It is clearly not that variety, but its identity is still in doubt.

§ The apple designated as Hort. No. 4941 was received under the name of Herschel Cox. The fruit differs in so many respects from the available descriptions of that variety that its identity is in doubt. It is therefore designated by the serial number given it at its receipt by the Office of Horticulture.

|| The apple designated as Smith is not Smith Cider, but a local variety originating in Kansas and never introduced into the trade.

¶ The apple designated as Winter Smokehouse is practically indistinguishable in appearance from Smokehouse, but has shown consistent differences in chemical composition which are believed to warrant regarding it as distinct from that variety.

## ANALYTICAL RESULTS

All the analytical data obtained in the course of the work are presented together in Table 1. In order to examine these data for the presence or absence of any mass fluctuations in chemical composition occurring from year to year, it was necessary to make such analysis and rearrangement of the results as would permit comparison of the crop of any one year with that of all the other years. Such a rearrangement was complicated by the fact that while the analyses extended over 6 years, the varieties did not all fruit annually, so that the forms analyzed in any year consisted of groups analyzed in 6, 5, 4, and 3 years, respectively, of the possible 6. Each of the 5-year, 4-year, and 3-year groups had to be further subdivided into classes, each containing varieties which had been analyzed in the same years

in order that the comparisons made might be rigidly accurate. When this rearrangement of the data had been made, each of the various groups was examined with respect to the content of total sugars, sucrose, titratable acidity, and astringent constituents of its members in each of the years in which the particular group was subjected to analysis. In assembling the results, consideration was had for the fact that occurrence of large numbers of maximum or minimum values in 1 year is more significant when the results of 5 or 6 years are being compared than when results for 4 or 3 years only are in hand. The method of weighing the results is fully explained in the discussion of results as to total sugar content. Recapitulations of the detailed comparisons are presented in Table 2, total sugar; Table 3, titratable acidity; and Tables 4, 5, and 6, total astringency, tannins, and astringent nontannins. In the recapitulations the results obtained in the detailed comparisons of the various subgroups have been combined into single groups, but in the accompanying discussions they are considered separately.

#### TOTAL SUGAR CONTENT

The results of examination of the data for the several years with respect to the comparative total sugar content of the varieties analyzed in each year are summarized in Table 2. Such summation does not permit direct evaluation of the several years, since it contains results derived from comparison of periods of varying lengths, to which differing degrees of significance should be attached. In the case of groups analyzed for four or more years, the tendency of the crop toward attainment of high or low sugar content in any year is not adequately indicated by the number of the group having maximum or minimum values. The number of values standing next to maximum or minimum, as the case may be, also has significance as indicating the degree to which massing of values toward one end of the possible range has occurred. In the case of data covering only three years only maximum or minimum values can be considered as significant. In the comparisons which immediately follow, as well as in those made with respect to other constituents in subsequent sections, the ranking of each year has been determined by combining the number of maximum values in the 3-year groups with the number of maximum and next to maximum values in the 6-year, 5-year, and 4-year groups, and comparing the total, expressed as a percentage of the number analyzed in that year, with percentages for the other years arrived at in like manner. In the case of minimum values an identical method has been employed.

Examination of the data by the method indicated shows that in order of total sugar content 1923 stands first, with 150 out of 200 varieties, or 75 per cent, having maximum values in the 3-year group and maximum or next to maximum values in the other groups. The year 1921 is second with 44 out of 76, or 57.9 per cent; 1925 third, with 49 out of 120, or 40.8 per cent; 1922 fourth, with 64 out of 199, or 32.1 per cent; 1920 fifth, with 22 out of 69, or 31.8 per cent; and 1924 sixth, with 32 out of 190, or 16.8 per cent. That this ranking is justified is further indicated by examination of the distribution in rank of the remaining analyses. The year 1923 has very small percentages of minimum values in all the groups, and the number of next to minimum values in the 4-year, 5-year, and 6-year

groups are also small. The year 1924 has 64.5 per cent of the 3-year group, 51.2 per cent of the 4-year group, and 43.6 per cent of the 5-year group, showing minimum values for sugar. The number of next to minimum values in the 4-year and 5-year groups is also large. There is a very evident tendency toward the massing of varieties in maximum or second-place positions with respect to sugar in 1923, while 1924 shows a like massing in minimum or next to minimum positions.

TABLE 2.—Comparative ranking of apple crop, 1920–1925, with respect to total sugar content

Crop of—	Number of varieties compared	Number of times the crop designated ranked as specified in total sugar content					
		First (maximum)	Second	Third	Fourth	Fifth	Sixth
1920	6 years, 9	0	1	3	2	1	2
	5 years, 29	3	6	7	6	7	
	4 years, 18	3	6	5	4		
	3 years, 13	3	5	5			
1921	6 years, 9	2	1	2	2	0	2
	5 years, 33	11	10	7	3	2	
	4 years, 26	5	11	8	2		
	3 years, 8	4	0	4			
1922	6 years, 9	0	1	2	1	3	2
	5 years, 45	4	3	12	19	7	
	4 years, 81	14	26	22	19		
	3 years, 64	16	35	13			
1923	6 years, 9	5	3	0	1	0	0
	5 years, 45	22	12	6	1	4	
	4 years, 84	48	16	13	7		
	3 years, 62	44	11	7			
1924	6 years, 9	1	1	2	2	2	1
	5 years, 39	2	5	5	10	17	
	4 years, 80	7	11	21	41		
	3 years, 62	5	17	40			
1925	6 years, 9	1	2	0	1	3	2
	5 years, 34	3	10	8	7	6	
	4 years, 67	13	19	20	15		
	3 years, 10	1	5	4			

The year 1921 stands second to 1923 in percentage of varieties having high sugar content. The crop was considerably reduced in size by frosts occurring on March 29 and 30 and April 2 and 11. Abnormally high temperatures throughout March had initiated the development of fruit buds, and the result of the series of frosts was practically total destruction of the early-blooming varieties and considerable injury to the later ones. The distribution of the injury to the late-blooming varieties was somewhat unusual. Practically all the fruit on the trees of the outer rows on the southern and western sides of the orchard was killed. In the body of the orchard, damage was in many cases confined to the tops or southwest sides of the trees. On the remaining branches, damage when present was confined to the central buds of the clusters, with the result that half to three-fourths of the tree had a crop of normal quantity.

The literature contains very little definite information as to the effects of such local destruction of buds upon the composition of the crop on the fruiting portions of the tree. It was believed that some light upon this question might be obtained by making a careful survey of the orchard, estimating and noting the percentage and

distribution of the crop on the trees of each variety, and examining the analytical data for such indications as they might yield. In consequence, detailed notes upon the varieties listed for analysis were made in the orchard in June and again in September before picking began. The varieties taken for analysis were restricted to those having two-thirds or more of the tree in fruit and with 80 to 100 per cent of the normal load on the fruiting portions. For purposes of comparison, a number of varieties having half or more of the tree barren, but with a fair load on the fruiting portion, were also sampled. While it was anticipated that an effect of the larger area of barren branches might be apparent when these groups were compared, exhaustive comparisons do not show any evidence of such effect upon the composition of the fruit. This matter will be discussed more in detail in a subsequent section, but it may be stated here that the rank attained by the year 1921 in point of sugar content is attributable to the seasonal conditions during its development, not to reduction of the load of fruit on the trees by frost injury.

Of the total number of variety samples analyzed in 1921, 28.9 per cent had maximum and 28.9 per cent next to maximum sugar. Of the group of 33 fruiting in five years, 21 had highest or next to highest sugar; of the group of 26 fruiting in four years, 16 had highest or next to highest sugar. The small numbers of varieties occupying low positions in the various groups are also indicative of the very general mass tendency to attain rather high sugar content in this year.

The year 1922 ranks fourth in the number of varieties having maximum or next to maximum sugar, 32.71 per cent of the 199 varieties analyzed falling in this group. That the year ranked far below 1923 in the intensity of the climatic factors favoring large accumulations of sugar in the crop is indicated by several facts. Only 17 per cent of the 199 varieties analyzed in 1922 had maximum sugar, while 59.5 per cent of the 200 varieties analyzed in 1923 had the maximum. In 1922, 15 per cent had the next to maximum amount, while in 1923 only 21 per cent fell into this group, so that the mass tendency toward highest place is much more pronounced in 1923. Also, the totals in first place are made up largely from analyses for three years only. Of the 64 varieties in this group, 47 were analyzed only in 1922, 1923, and 1924. Of these 47 varieties, 32 had their maximum and 9 their next to maximum sugar in 1923, while 30 had their minimum and 11 their next to minimum sugar in 1924. Of the 47, 11 had maximum, 26 next to maximum, and 7 minimum in 1922. The effect of comparing 1922 with the exceptionally unfavorable year 1924 and the highly favorable year 1923 results in throwing a large number of the group into middle position. The fact that a considerable number of the varieties bore a crop of less than normal size in 1921, and that the crop of 1922 consequently followed a year of fairly vigorous vegetative growth may also contribute in some degree toward the development of higher sugar content than would have been found under identical conditions after a year of normal bearing.

With all these considerations in mind, 1922 must be ranked as decidedly inferior to 1923, in so far as conditions favoring maximum accumulation of sugar in fruits are concerned. As compared with 1921, it has a very much smaller percentage of varieties attaining

highest sugar content—17.9 as against 28.9 per cent, and also a smaller percentage in second place—15 as against 28.9 per cent. The very considerable number of varieties ranking in third and fourth place in 1922 also justifies the placing of 1921 somewhat ahead of 1922 in respect to sugar content of crop.

The year 1925 ranks third in point of number of varieties attaining high sugar content. The total number of varieties analyzed was 120. The reduction in number was due to the fact that the year was one of rather light bearing throughout the variety orchard, and a large number of varieties used in previous years either failed to set fruit or had so small a crop that samples would not have been representative. A few varieties which had crops of normal size, but which had fruited only occasionally in previous years, were not sampled because of the limited time available for the work. The 120 varieties included in the analyses in all cases had crops which approximated in quantity those borne by the same trees in previous years of the work. This year is like 1924 in that the number of varieties having maximum sugar content is small, 15 per cent, or about half the percentage found in first place in 1921. It owes its rank to the possession of 25.8 per cent in next to maximum place. Inspection of the distribution of results over the groups (Table 2) shows that the year is characterized by the absence of extremes and the massing of the results in second, third, and fourth places. It ranks third in the series of six years in point of number of varieties attaining high sugar content.

The year 1920 ranks fifth in percentage of varieties having maximum or next to maximum sugar content. Of the 69 varieties examined, 9, or 13.03 per cent, had maximum, and 13, or 18.8 per cent, next to maximum values, or 31.8 per cent of first and second place values combined. This percentage is almost identical with the 32.1 per cent of similar values found for 1922, but the fact that second-place values enter more largely into the total would rank the year as slightly below 1922 in this respect. The fact that it has considerably larger percentages of minimum values in all the groups would confirm this judgment. It is very considerably superior to 1924, since there is no such pronounced tendency toward large numbers of minimum values as is manifested in that year.

#### RELATION OF SUCROSE CONTENT TO TOTAL SUGAR CONTENT

The amounts of sucrose present in the fruit and the ratio borne by the sucrose content to the total sugar present show considerable variations from year to year, but there is a rather consistent tendency for sucrose and total sugar to attain maximum or minimum values together. Detailed consideration of this point is apart from the purpose of the present discussion, but it may be pointed out that in 1920, of 69 varieties analyzed, 9 had maximum total sugar, 2 had maximum sucrose; in 1921, of 76 varieties analyzed, the numbers were 22 and 20, respectively; in 1922, of 199 varieties analyzed, 34 and 35; in 1923, of 200 varieties, 119 and 122; in 1924, of 190 varieties, 15 and 21; and in 1925, of 120 varieties, 18 and 17. There is somewhat less close agreement in the occurrence of minimum values of total sugar and sucrose, but in no case are maximum or next to maximum values for one associated with minimum values for the other. It is therefore apparent that conditions which are favorable to the attainment of high total sugar content are favorable also to

the attainment of a high content of sucrose. Conditions which depress sugar storage in the fruit result also in a low content of sucrose. The relationship is a general rather than an absolute one, but it is rather consistently sustained throughout the results.

#### TOTAL TITRATABLE ACIDITY

Table 3 presents a recapitulation of results with respect to titratable acidity, made by the method employed in summarizing the results upon total sugars. The year 1921 leads in percentage of varieties having high acidity, with 46.0 of maximum and 21.1 per cent of next to maximum values for acid constituents, or 67.1 per cent together. The year 1920 has 56.5 per cent of high values, of which 23.2 per cent are maximum and 33.3 per cent next to maximum values; 1923 has 55.8 per cent, of which 36 per cent are maximum and 19.9 per cent next to maximum values; and 1922 has 52.7 per cent, of which 36.1 per cent are maximum and 16.5 per cent next to maximum values. These years rank so close together that assignment of relative place can be made only by considering them in some detail. Since 1923 and 1922 each has 36 per cent of maximum values, they clearly rank higher than 1920, which has 23.2 only. As 1923 has 19.9 per cent of next to maximum values as compared with 16.5 per cent of such values for 1922, 1923 apparently ranks slightly above 1922 with respect to acidity. Comparison of the numbers of minimum or next to minimum values for the two years confirms this ranking. The year 1920 ranks fourth for the reason that it has a much smaller number of maximum and a considerably larger number of next to maximum acidity values than are found in either 1922 or 1923. The differences between the three years are, however, very slight.

TABLE 3.—Comparative ranking of apple crop, 1920-1925, with respect to acid content

Crop of—	Number of varieties compared	Number of times the crop designated ranked as specified in acid content					
		First (maximum)	Second	Third	Fourth	Fifth	Sixth
1920	6 years, 9	1	3	3	1	1	0
	5 years, 29	8	11	5	3	2	
	4 years, 18	5	9	4	0		
	3 years, 13	2	5	6			
1921	6 years, 9	5	1	2	1	0	0
	5 years, 33	14	6	10	2	1	
	4 years, 26	12	9	2	3		
	3 years, 8	4	2	2			
1922	6 years, 9	2	0	2	4	1	0
	5 years, 45	8	13	13	9	2	
	4 years, 81	30	20	23	8		
	3 years, 64	32	20	12			
1923	6 years, 9	1	4	1	0	2	1
	5 years, 45	11	9	8	12	5	
	4 years, 84	32	26	22	4		
	3 years, 62	28	23	11			
1924	6 years, 9	0	1	1	1	3	3
	5 years, 39	4	6	7	15	7	
	4 years, 80	9	24	24	23		
	3 years, 62	7	20	35			
1925	6 years, 9	0	0	0	2	2	5
	5 years, 34	0	0	3	4	27	
	4 years, 67	1	1	14	51		
	3 years, 10	0	3	7			



The situation is decidedly otherwise in 1924, which ranks fifth in point of acid content. The year has 10.5 per cent of varieties with maximum acid, 46.3 per cent with next to maximum. There is a decided preponderance of minimum results. Of the varieties analyzed in 5 years, 56.4 per cent have minimum or next to minimum acidity; of those analyzed 4 years, 58.7 per cent take similar position; while of those analyzed 3 years only, 56.4 per cent had their minimum in 1924. In each of the groups there is the same general situation—a small number of varieties high in acidity, a somewhat larger number ranking as medium or average, and a majority with acid content at or near the minimum.

In 1925 the same tendency is shown in a much more pronounced degree. Less than 2 per cent of the varieties had maximum or next to maximum acidity, while in the groups analyzed for different periods 70 to 80 per cent of each group show minimum acidity. The mass tendency toward low acidity observable in 1924 manifests itself in extreme degree in 1925. The two years 1924 and 1925 therefore differ only in the intensity of a mass effect exactly opposite to that seen in the other years.

#### TANNIN AND OTHER ASTRINGENT SUBSTANCES

It is a matter of great regret that determinations of total astringency and of astringent nontannins were not made upon the freshly pressed juices in 1920 and 1921. Immediate complete chemical analysis of the juices as they were pressed was impossible in these years, as the time of the writer was largely occupied with other investigations which necessitated absence from Washington for a part of the time during which samples were being taken. The determinations made upon the fresh juices were therefore restricted to free reducing sugars, total sugars, titratable acidity, and, in part, total solids. Samples in all cases were preserved by Pasteurization, and it was planned to employ these in a study of astringent substances to be made at a later time. While it was known that such treatment would bring about some alterations in the astringent substances in solution in the juice, the writer in common with earlier workers, erred in regarding these as of minor importance. It was not until the beginning of a study of the effects of Pasteurization upon the composition of apple and grape juices, late in 1921, that the extent and importance of the alterations in astringent substances produced by Pasteurizing a fruit juice were realized.<sup>3</sup> These changes affect both true tannins and astringent nontannins, and are conditioned upon so many factors that determinations of these constituents made upon Pasteurized juices give no indication as to what their amounts may have been in the same juices prior to heating. These facts were discovered so late in the season of 1921 that it was not possible to make astringency determinations upon more than a small number of fresh juices in that year. Such determinations were made as a part of all analyses in subsequent years. It is believed that the data for the four years 1922–1925 are sufficient in amount and were accumulated over a period of years exhibiting a sufficiently varied range of seasonal conditions to warrant the drawing of some general conclusions.

<sup>3</sup> CALDWELL, J. S. THE PHYSICAL AND CHEMICAL CHANGES OCCURRING IN FRUIT JUICES AS A RESULT OF PASTEURIZATION. [Unpublished manuscript.]

The term "total astringent substances" is a broadly inclusive one, embracing all substances which reduce potassium permanganate under definite conditions. True tannins, tannin derivatives, coloring matters, and substances of highly diverse and partially unknown character are included in the determination. A second titration with potassium permanganate after precipitation and removal of the true tannins with gelatin permits a determination of the tannins by difference and an estimation of the amount of nontannins. Both are then expressed in terms of tannin by the use of a conventional factor (5). Both the method of determination and that of expression of the results are exceedingly unsatisfactory. It is known that the oxidation of oxidizable substances is far from complete under the usual conditions of the test (16), that the application of the conventional factor to the tannins of fruits gives a result which is only about 60 per cent of the true one (23), and that in the case of certain red wines the use of the conventional factor to express nontannins gives a result which is only 30 per cent of the true weight of these substances (29). In spite of these defects, no method which is at once more accurate and equally easy of application has been developed to replace the modified Loewenthal procedure. That method has consequently been employed, as the results obtained by its use have value for purposes of comparison despite their lack of quantitative accuracy.

If the sum total of astringent materials is affected in a definite manner by climatic conditions it is a matter of some practical importance, since the palatability of a fruit or the beverage quality of a juice made therefrom depends upon the relative proportions of sugar, acids, and astringent materials present. In determining the effect upon the palate, all the astringent substances present play a part, no matter what their origin or chemical relationships. If climatic conditions exercise such a decisive effect upon some one or more of these constituents as to influence the sum total in a definite direction, the palatability of the juice will be affected. It is as important from the practical point of view to know whether a given set of climatic conditions tends to increase or decrease total astringency as it is to know their effect upon sugar and acid content, since an alteration in the amount of any one of these constituents modifies the ratios between them and alters their collective effect upon the palate. That climatic conditions have such a definite effect upon total astringency in the case of the grape has been shown by Caldwell (11).

Comparison of the summaries on total astringency, tannin, and astringent nontannins in Tables 4, 5, and 6 show no evidence that total astringency is definitely affected as regards its absolute amount by seasonal conditions. In 83 varieties on which determinations were made annually in 1922-1925 (Table 4) there is no indication of a mass tendency toward high or low total astringency in any year. In the group of 74 varieties analyzed only in the three years 1922-1924 there appears to be some tendency toward high astringency in 1923 and toward low in 1924, but 1922 has larger numbers of maximum and minimum results than of second-place results. The various smaller groups also show no evidence of concentration of high or low values in any one year. It is evident that total astringency does not display mass behavior in its variations in absolute amount from year to year and that these variations can not be directly related to differences in the seasonal conditions of the year concerned.

TABLE 4.—Comparative ranking of apple crop, 1922-1925, with respect to total astringency

Crop of—	Years analyzed	Number of varieties	Number of times the crop designated ranked as specified in total astringency			
			First (maximum)	Second	Third	Fourth
1922-----	4 years-----	83	22	21	15	25
	3 years (1922, 1923, 1924)-----	74	27	20	27	-----
	3 years (1922, 1923, 1925)-----	14	6	1	7	-----
	3 years (1922, 1924, 1925)-----	7	4	2	1	-----
1923-----	4 years-----	83	22	21	21	19
	3 years (1922, 1923, 1924)-----	74	30	28	16	-----
	3 years (1922, 1923, 1925)-----	14	3	7	4	-----
	3 years (1923, 1924, 1925)-----	10	5	3	2	-----
1924-----	4 years-----	83	19	20	24	20
	3 years (1922, 1923, 1924)-----	74	17	26	31	-----
	3 years (1922, 1924, 1925)-----	7	1	4	2	-----
	3 years (1923, 1924, 1925)-----	10	0	5	5	-----
1925-----	4 years-----	83	20	22	23	18
	3 years (1922, 1923, 1925)-----	14	5	9	0	-----
	3 years (1922, 1924, 1925)-----	7	2	2	3	-----
	3 years (1923, 1924, 1925)-----	10	5	3	2	-----

## TRUE TANNINS

A somewhat unexpected fact in respect to the portion of the astringent material which is precipitable by addition of gelatin-salt solution is that in practically all varieties there is a very wide variation from year to year in the amounts found at like stages of maturity. In many cases the variation exceeds 100 per cent. These extremely wide variations occur not only in highly astringent crab varieties such as Amère du Surville, Entz, Hyslop, Julian de Paulmier, Soulard Crab, and Red Siberian, but also in apple varieties such as Hubbards-ton, Northwestern Greening, Nero, Mother, Shoemaker, and Rome Beauty. In a much smaller group of crab varieties which also contains a few rather highly astringent fruits, such as Florence, Lyman Prolific, and Yellow Siberian, with a large number of apples such as Allington Pippin, Winter Banana, Fameuse, Sutton, Cox No. 13, Roxbury Russet, and White Pearmain, the variations in true tannins are of relatively smaller magnitude, from 10 to 50 per cent. The number showing this degree of constancy is small as compared with the number of those displaying a wide range.

As indicated in Table 5, the data available upon true tannins are derived from a group of 83 varieties fruiting annually in the four years 1922-1925, a group of 74 fruiting in the three years 1922-1924 but having no crop or else crops too small to be included in the analyses in 1925, and three small groups each of which fruited in three of the four years.

Combining the numbers of maximum values in the 3-year groups with the maximum and next to maximum values of the 4-year group, the percentages in the several years are as follows: For 1923, 53.5; for 1925, 42.1; for 1924, 36.2; and for 1922, 35.3 per cent. This would rank the years in the order named in point of astringent nontannin content but with no significant difference between 1924 and 1922. Considering the data in greater detail, it is found that of the group of 83 varieties analyzed in four years,

1922, 1923, and 1925 each have 22 with maximum values, while 1924 has 17. When maximum and next to maximum values are combined, 1923 has 52 as against 39 for 1925, 38 for 1924, and 37 for 1922. The results for the group of 74 varieties analyzed in the years 1922-1924 rank these years in the order 1923 first, 1924 second, and 1922 third. Comparison of the numbers of minimum values give the three years the same rank. The numbers in the small groups which fruited in 1925 but failed to fruit in one of the other years are too small to be of much significance; they indicate that the year was one of rather low true tannin content.

TABLE 5.—Comparative ranking of apple crop, 1922-1925, with respect to true tannin

Crop of—	Number of varieties compared	Number of times the crop designated ranked as specified in true tannins			
		First (maximum)	Second	Thurd	Fourth
1922	4 years, 83	22	15	14	32
	3 years (1922, 1923, 1924), 74	16	23	35	
	3 years (1922, 1923, 1925), 14	7	1	6	
	3 years (1922, 1924, 1925), 7	3	1	3	
1923	4 years, 83	22	30	17	14
	3 years (1922, 1923, 1924), 74	35	19	17	
	3 years (1922, 1923, 1925), 14	4	2	8	
	3 years (1923, 1924, 1925), 10	3	4	3	
1924	4 years, 83	17	21	23	22
	3 years (1922, 1923, 1924), 74	20	32	22	
	3 years (1923, 1924, 1925), 10	3	4	3	
	3 years (1922, 1924, 1925), 7	2	3	2	
1925	4 years, 83	22	17	30	14
	3 years (1923, 1924, 1925), 10	4	2	4	
	3 years (1922, 1924, 1925), 7	2	3	2	
	3 years (1922, 1923, 1925), 14	3	11	0	

These comparisons appear to justify the conclusion that 1923 ranked distinctly above any of the other years for which data are available in respect to true tannins present in the crop. The remaining years show no outstanding differences which entitle one to be ranked higher than another. There is no year showing a distinct minimum, and the evidence scarcely justifies one in going further than to say that 1923 had a distinctly higher content of true tannins in the crop than was found to be present in the other years. If the tannin content of the crop as a whole is affected by climatic conditions, the effect is very much less pronounced in character than that exerted upon other constituents.

#### ASTRINGENT NONTANNINS

In the group of 83 varieties analyzed in the four years 1922 to 1925 (Table 6), 1924 has the largest percentage of maximum astringent nontannins, namely 31.3 per cent, but is rather closely followed by 1922 with 28.9 per cent. The years 1925 and 1923 rank considerably lower, with percentages of 20.4 and 19.2 maximum values, respectively. Combination of maximum and second-place values for each of the years would rank them in the order 1924, 1922, 1925, and 1923. The results with the group of 74 varieties analyzed in 1922 to 1924 only

are quite different, since 1922 has 52.7 per cent maximum values as compared with 33.8 per cent for 1923 and 14.8 per cent for 1924. Since the results with the 4-year and the 3-year groups are quite out of agreement, no conclusion can be drawn other than that there is evidently no consistent mass tendency toward maximum or minimum astringent nontannin content in any of the several years considered.

TABLE 6.—*Comparative ranking of apple crop, 1922–1925, with respect to astringent nontannins*

Crop of—	Number of varieties compared	Number of times the crop designated ranked as specified in astringent nontannins			
		First (maximum)	Second	Third	Fourth
1922	4 years, 83	24	17	13	29
	3 years (1922, 1923, 1924), 74	39	12	23	-----
	3 years (1922, 1923, 1925), 14	7	2	5	-----
	3 years (1922, 1924, 1925), 7	4	1	2	-----
1923	4 years, 83	16	21	27	19
	3 years (1922, 1923, 1924), 74	25	35	14	-----
	3 years (1922, 1923, 1925), 14	2	6	6	-----
	3 years (1923, 1924, 1925), 10	5	4	1	-----
1924	4 years, 83	26	21	19	17
	3 years (1922, 1923, 1924), 74	11	28	35	-----
	3 years (1923, 1924, 1925), 10	2	3	5	-----
	3 years (1922, 1924, 1925), 7	2	3	2	-----
1925	4 years, 83	17	24	25	17
	3 years (1923, 1924, 1925), 10	3	3	4	-----
	3 years (1922, 1924, 1925), 7	1	3	3	-----
	3 years (1922, 1923, 1925), 14	5	6	3	-----

It may be pointed out that the rankings of the years with respect to true tannins and astringent nontannins show no consistent relations. The year 1923 had a high content of true tannins, but this is not clearly associated with a low content of nontannins, or the reverse. The two constituents appear to fluctuate independently, with no outstanding mass tendencies which could be attributed to the effect of varying seasonal conditions apparent in the case of either. In consequence, total astringency, which is the sum of the two, shows no indication of mass behavior in its variation in absolute amount from year to year.

#### ACID-ASTRINGENCY-SUGAR RATIO

As has been pointed out in a preceding section, palatability of a fruit or fruit juice depends upon the relative proportion of acids, astringent materials, and sugar simultaneously presented to the nerve endings of taste. If the proportion between amounts of these constituents is kept constant, their absolute amounts may be varied over a considerable range without producing any effect discernible by the sense of taste. On the other hand, very slight differences in the proportion of the different constituents are readily perceived, as is shown by the fact that our judgments that one juice is more astringent but less sweet than another, or that one fruit is sweeter than another, are based upon small chemical differences. The error of such judgments results chiefly from the fact that higher or lower sugar con-

tent is frequently confused with decreased or increased acid content. In so far as our judgments of dessert quality in fruit are based upon characters measurable by chemical analysis, they are determined by the ratio between acid, tannin, and sugar present in the flesh. In the case of a juice, this ratio, plus the effects of the minute amounts of volatile substances which give characteristic individual aroma and flavor, determines our verdict as to quality, since considerations as to physical characters, such as texture and crispness of flesh, do not enter as they do in the case of a fruit.

For this reason the proportion borne by the sugars, astringent materials, and acids one to another is an exceedingly important factor in determining palatability whether in a fruit or in a fruit juice. It follows that it is a matter of great practical importance to ascertain the amount of annual variation in this ratio in apple varieties and to learn whether any causal relations exist between climatic conditions and such variations.

While the amount of data here available is not such as to warrant dogmatic conclusions, it may give some indications as to what may be expected as a result of further investigation.

TABLE 7.—*Acid-astringency-sugar ratios for varieties of apples analyzed in years shown*

Variety	1922	1923	1924	1925
Four years' analyses:				
Abernathy.....	1.0.22 : 21.7	1.0.42 : 27.6	1:0.24 : 29.0	1:0.39 : 34.5
Algérienne.....	1: .72 : 26.1	1:1.13 : 52.2	1: .83 : 40.1	1:1.04 : 79.6
Allington Pippin.....	1: .12 : 12.2	1: .22 : 18.2	1: .14 : 13.5	1: .31 : 27.2
Amère du Surville.....	1: .82 : 24.2	1:3.49 : 45.8	1:2.95 : 74.2	1:2.69 : 73.2
Akin.....	1: .25 : 24.2	1: .24 : 32.3	1: .21 : 23.9	1: .25 : 35.9
Arctic.....	1: .26 : 33.7	1: .27 : 22.9	1: .31 : 19.2	1: .35 : 29.1
Babbitt.....	1: .092 : 8.4	1: .054 : 9.3	1: .109 : 9.5	1: .11 : 11.9
Baker.....	1: .14 : 20.2	1: .20 : 31.7	1: .20 : 21.9	1: .25 : 28.4
Baldwin.....	1: .15 : 18.4	1: .21 : 23.5	1: .29 : 20.7	1: .22 : 24.0
Ben Hur.....	1: .21 : 23.0	1: .21 : 31.1	1: .21 : 30.3	1: .42 : 52.7
Bennes.....	1: .20 : 29.0	1: .23 : 33.9	1: .34 : 38.7	1: .31 : 32.7
Bethlehemite.....	1: .22 : 35.8	1: .24 : 30.7	1: .29 : 31.6	1: .63 : 66.1
Black Ben.....	1: .25 : 15.3	1: .24 : 22.8	1: .25 : 24.8	1: .21 : 20.6
Bughorn.....	1: .31 : 20.1	1: .19 : 20.9	1: .23 : 15.9	1: .37 : 28.4
Carson.....	1: .21 : 16.6	1: .11 : 15.9	1: .071 : 12.1	1: .11 : 20.2
Celestia.....	1: .16 : 17.8	1: .11 : 19.8	1: .15 : 29.8	1: .22 : 31.1
Collins.....	1: .13 : 13.3	1: .14 : 18.7	1: .11 : 18.2	1: .28 : 27.9
Cox No. 13.....	1: .099 : 21.6	1: .12 : 20.5	1: .13 : 26.1	1: .21 : 33.7
Dixon.....	1: .23 : 29.0	1: .12 : 16.2	1: .095 : 18.6	1: .17 : 23.9
Domine.....	1: .22 : 23.3	1: .21 : 25.2	1: .143 : 22.2	1: .247 : 31.9
Pyrus angustifolia.....	1: .29 : 1.39	1: .46 : 2.76	1: .27 : 1.77	1: .39 : 2.02
Flory.....	1: .12 : 10.3	1: .13 : 13.5	1: .22 : 15.3	1: .16 : 11.1
Greenville.....	1: .25 : 23.1	1: .12 : 16.2	1: .14 : 15.2	1: .24 : 24.9
Haas.....	1: .11 : 10.8	1: .19 : 21.1	1: .20 : 14.8	1: .27 : 22.7
Hagloe Crab.....	1: .123 : 6.78	1: .19 : 17.1	1: .21 : 14.3	1: .21 : 17.0
Hilaire.....	1: .18 : 13.5	1: .12 : 13.3	1: .17 : 13.3	1: .21 : 23.4
Hoover.....	1: .13 : 15.9	1: .14 : 15.9	1: .22 : 15.9	1: .21 : 24.8
Huntsman.....	1: .35 : 38.1	1: .18 : 25.9	1: .165 : 21.6	1: .33 : 35.1
Hyslop.....	1: .56 : 15.4	1: .61 : 22.0	1: .65 : 16.8	1: .61 : 26.1
Hort. No. 4941.....	1: .81 : 71.0	1: .43 : 44.7	1: .79 : 85.6	1: .85 : 80.7
Indian.....	1: .30 : 21.8	1: .18 : 18.7	1: .40 : 26.3	1: .40 : 30.0
Jeffers.....	1: .18 : 10.5	1: .18 : 20.8	1: .30 : 33.0	1: .20 : 23.1
Keeper.....	1: .11 : 21.1	1: .13 : 28.6	1: .12 : 24.0	1: .13 : 26.4
Kentucky Sweet.....	1: .74 : 36.7	1: .1.09 : 65.0	1: .82 : 65.2	1: .79 : 59.6
Klickitat.....	1: .14 : 19.2	1: .20 : 35.6	1: .20 : 24.7	1: .29 : 35.4
Kooroochiang.....	1: .072 : 10.3	1: .96 : 11.7	1: .12 : 11.5	1: .18 : 21.1
Magg.....	1: .117 : 19.4	1: .24 : 22.3	1: .21 : 15.0	1: .27 : 26.5
Magog.....	1: .20 : 25.6	1: .15 : 18.8	1: .30 : 14.1	1: .18 : 19.4
Monmouth.....	1: .12 : 17.6	1: .22 : 27.9	1: .23 : 19.4	1: .33 : 36.9
Mann.....	1: .19 : 20.6	1: .16 : 19.7	1: .19 : 17.9	1: .32 : 32.1
Martin.....	1: .29 : 39.3	1: .38 : 61.3	1: .27 : 45.1	1: .53 : 55.0
McAfee.....	1: .21 : 19.9	1: .30 : 30.4	1: .30 : 25.5	1: .51 : 43.5
McIntosh.....	1: .17 : 16.6	1: .19 : 17.5	1: .17 : 24.8	1: .28 : 25.4
McMahon.....	1: .14 : 7.6	1: .085 : 8.1	1: .12 : 10.2	1: .17 : 17.2
Menagere.....	1: .23 : 25.4	1: .25 : 31.9	1: .18 : 25.8	1: .43 : 41.5
Missouri.....	1: .24 : 22.2	1: .13 : 17.3	1: .16 : 20.3	1: .28 : 26.8
Mother.....	1: .30 : 31.8	1: .26 : 26.8	1: .25 : 23.2	1: .46 : 45.9

TABLE 7.—*Acid-astringency-sugar ratios for varieties of apples analyzed in years shown—Continued*

Variety	1922	1923	1924	1925
Four years' analyses—Continued.				
Nero.....	1:0.23 : 33.1	1:0.36 : 39.2	1:0.44 : 36.5	1:0.45 : 39.0
Newtown Spitzenburg.....	1: .15 : 19.9	1: .23 : 30.8	1: .21 : 24.3	1: .28 : 36.6
Nickajack.....	1: .19 : 21.4	1: .28 : 41.7	1: .242 : 37.1	1: .52 : 42.9
Ohio Pippin.....	1: .21 : 20.4	1: .27 : 23.4	1: .25 : 29.8	1: .23 : 24.5
Pawpaw.....	1: .19 : 17.9	1: .12 : 14.4	1: .15 : 14.6	1: .24 : 25.1
Peron.....	1: .18 : 10.2	1: .21 : 17.6	1: .27 : 18.5	1: .45 : 30.7
Rabum.....	1: .47 : 51.1	1: .27 : 32.2	1: .35 : 32.6	1: .28 : 28.6
Ralls.....	1: .13 : 18.6	1: .17 : 27.8	1: .18 : 29.6	1: .33 : 39.4
Rome Beauty.....	1: .24 : 28.9	1: .18 : 30.9	1: .31 : 31.1	1: .36 : 31.9
Ronk.....	1: .22 : 19.3	1: .12 : 18.1	1: .19 : 16.2	1: .19 : 21.2
Salome.....	1: .071 : 15.9	1: .12 : 19.8	1: .12 : 13.7	1: .192 : 25.3
Scott Winter.....	1: .08 : 9.8	1: .11 : 10.9	1: .105 : 16.9	1: .15 : 13.3
Shackleford.....	1: .13 : 18.1	1: .19 : 24.2	1: .23 : 21.8	1: .41 : 42.0
Shoemaker.....	1: .187 : 13.7	1: .14 : 11.5	1: .41 : 26.4	1: .29 : 25.4
Shone.....	1: .095 : 22.8	1: .12 : 21.2	1: .14 : 22.0	1: .17 : 30.7
Smokehouse.....	1: .12 : 19.3	1: .21 : 23.4	1: .23 : 20.0	1: .32 : 31.6
Swaar.....	1: .18 : 19.7	1: .14 : 21.7	1: .16 : 16.6	1: .16 : 28.7
Sweet Orange.....	1: .64 : 41.6	1: .22 : 25.3	1: .51 : 77.0	1: .67 : 61.2
Sutton.....	1: .23 : 22.2	1: .31 : 32.2	1: .16 : 23.3	1: .30 : 32.7
Tompkins King.....	1: .18 : 19.2	1: .27 : 31.0	1: .24 : 23.6	1: .31 : 32.3
Transcendent.....	1: .31 : 8.8	1: .48 : 15.2	1: .76 : 21.1	1: .27 : 13.0
Vandevere.....	1: .13 : 19.7	1: .21 : 23.7	1: .19 : 21.9	1: .22 : 33.8
Vanderpool.....	1: .22 : 33.5	1: .27 : 24.0	1: .32 : 37.6	1: .49 : 44.5
Victoria Sweet.....	1: .20 : 25.5	1: .51 : 43.2	1: .66 : 63.6	1: .97 : 89.3
Walbridge.....	1: .15 : 14.1	1: .19 : 24.9	1: .18 : 15.4	1: .23 : 19.4
Wallace Howard.....	1: .41 : 32.0	1: .30 : 31.6	1: .24 : 22.3	1: .36 : 38.9
Weaver.....	1: .64 : 54.4	1: .52 : 52.9	1: .52 : 56.2	1: .72 : 66.0
Wells.....	1: .19 : 24.2	1: .20 : 26.3	1: .091 : 13.5	1: .18 : 20.4
Westfield.....	1: .24 : 22.7	1: .22 : 26.4	1: .19 : 23.3	1: .28 : 29.3
Wingate.....	1: .17 : 10.7	1: .21 : 17.0	1: .24 : 19.7	1: .21 : 19.0
Winter Paradise.....	1: .127 : 89.1	1: .108 : 92.2	1: .156 : 88.0	1: .176 : 124.0
Wolf River.....	1: .097 : 9.5	1: .13 : 13.0	1: .13 : 13.6	1: .13 : 15.5
Yellow Transparent.....	1: .28 : 11.6	1: .16 : 6.73	1: .31 : 13.8	1: .15 : 15.7
Yopp.....	1: .28 : 32.0	1: .25 : 33.4	1: .34 : 23.9	1: .33 : 32.2
Three years' analyses:				
Alton.....	1: .121 : 16.4	1: .126 : 23.5	1: .085 : 15.0	.....
Arnold.....	1: .16 : 20.5	1: .15 : 13.6	1: .20 : 22.0	.....
Baltimore.....	1: .50 : 42.4	1: .23 : 33.7	1: .39 : 34.3	.....
Barry.....	1: .10 : 6.2	1: .13 : 9.6	1: .12 : 9.9	.....
Black Annette.....	1: .24 : 25.0	1: .17 : 21.6	1: .32 : 28.9	.....
Blenheim.....	1: .10 : 18.1	1: .167 : 16.2	1: .16 : 15.0	.....
Boiken.....	1: .083 : 12.6	1: .12 : 14.9	1: .081 : 12.0	.....
Brackett.....	1: .34 : 29.4	1: .23 : 25.4	1: .27 : 26.2	.....
Brown.....	1: .205 : 21.6	1: .19 : 23.9	1: .17 : 18.2	.....
Buckingham.....	1: .10 : 22.5	1: .22 : 21.1	1: .22 : 20.8	.....
Buckskin.....	1: .15 : 17.3	1: .17 : 20.3	1: .26 : 18.0	.....
Canada Reinette.....	1: .19 : 18.5	1: .12 : 12.0	1: .21 : 15.7	.....
Clayton.....	1: .31 : 35.7	1: .14 : 16.3	1: .30 : 29.3	.....
Colvert.....	1: .34 : 44.5	1: .35 : 36.4	1: .28 : 26.8	.....
Cotter.....	1: .12 : 19.2	1: .13 : 22.8	1: .20 : 21.7	.....
Covert.....	1: .20 : 14.1	1: .34 : 21.7	1: .22 : 13.8	.....
Cox No. 11.....	1: .15 : 15.8	1: .078 : 9.6	1: .06 : 11.8	.....
Cross.....	1: .29 : 17.5	1: .43 : 31.3	1: .30 : 21.7	.....
Daniel.....	1: .21 : 19.1	1: .22 : 23.6	1: .28 : 35.5	.....
Doctor.....	1: .069 : 15.4	1: .16 : 22.4	1: .11 : 18.9	.....
Dudley.....	1: .12 : 8.5	1: .16 : 10.7	1: .14 : 14.0	.....
Early Richmond.....	1: .10 : 9.98	1: .076 : 6.55	1: .088 : 7.8	.....
Early Ripe.....	1: .17 : 10.5	1: .117 : 11.0	1: .13 : 9.02	.....
Entz.....	1: .31 : 8.7	1: .20 : 5.7	1: .30 : 6.7	.....
Evening Party.....	1: .276 : 30.3	1: .46 : 52.1	1: .35 : 31.9	.....
Fallowater.....	1: .51 : 41.8	1: .18 : 28.7	1: .32 : 38.1	.....
Family.....	1: .092 : 9.43	1: .104 : 13.8	1: .102 : 13.5	.....
Golden Pippin.....	1: .15 : 13.4	1: .16 : 16.7	1: .28 : 25.5	.....
Golden Russet.....	1: .18 : 25.9	1: .16 : 21.4	1: .18 : 23.4	.....
Granny Smith.....	1: .14 : 21.2	1: .18 : 19.7	1: .16 : 15.8	.....
Grosh.....	1: .15 : 34.1	1: .25 : 25.2	1: .20 : 20.0	.....
Heidemeyer.....	1: .175 : 19.4	1: .24 : 22.9	1: .24 : 20.8	.....
Henry Clay.....	1: .15 : 7.8	1: .205 : 14.9	1: .28 : 17.6	.....
Hogg.....	1: .13 : 6.7	1: .068 : 6.08	1: .11 : 9.4	.....
Indiana Favorite.....	1: .23 : 27.7	1: .23 : 39.0	1: .17 : 32.7	.....
Jersey Sweet.....	1: .86 : 60.3	1: .116 : 88.0	1: .106 : 128.0	.....
Lawver.....	1: .13 : 18.0	1: .13 : 16.1	1: .25 : 20.0	.....
Lou.....	1: .094 : 8.9	1: .06 : 5.2	1: .14 : 10.6	.....
Melo Gelato.....	1: .30 : 37.1	1: .60 : 49.3	1: .45 : 46.0	.....
Morris Red.....	1: .27 : 39.5	1: .21 : 26.5	1: .47 : 42.1	.....
Muster.....	1: .34 : 30.2	1: .33 : 28.6	1: .67 : 47.6	.....
Oakland.....	1: .31 : 41.1	1: .36 : 43.1	1: .21 : 23.6	.....
Ortley.....	1: .12 : 17.7	1: .11 : 15.3	1: .178 : 24.8	.....
Ozone.....	1: .364 : 30.9	1: .17 : 23.6	1: .17 : 15.3	.....
Pifer.....	1: .27 : 32.0	1: .25 : 31.4	1: .34 : 39.3	.....
Piper.....	1: .28 : 18.3	1: .37 : 15.2	1: .38 : 19.2	.....

TABLE 7.—*Acid-astringency-sugar ratios for varieties of apples analyzed in years shown—Continued*

Variety	1922	1923	1924	1925
Three years' analyses—Continued.				
Primate.....	1:0.27 :20.3	1:0.16 :11.1	1:0.053: 57.0	-----
Rebel.....	1: .33 :41.8	1: .16 :30.0	1: .23 : 28.7	-----
Red June.....	1: .28 :14.2	1: .20 :13.1	1: .33 : 28.4	-----
Red Siberian.....	1: .39 : 9.7	1: .703:13.8	1: .90: 15.0	-----
Reynard.....	1: .11 :14.5	1: .17 :17.5	1: .18 : 21.8	-----
Ruby Gem.....	1: .21 :10.7	1: .25 :16.1	1: .293: 21.2	-----
Sierra Beauty.....	1: .08 :17.6	1: .115:18.8	1: .11 : 14.1	-----
Smith Cider.....	1: .22 :21.2	1: .25 :26.3	1: .30 : 28.9	-----
Spencer Seedling.....	1: .307:35.5	1: .48 :46.4	1: .41 :43.4	-----
Springdale.....	1: .66 :52.7	1: .27 :22.8	1: .39 : 36.1	-----
Stanard.....	1: .13 :17.3	1: .31 :25.3	1: .30 : 22.3	-----
Stark.....	1: .15 :25.7	1: .20 :28.7	1: .30 : 32.1	-----
Starkey.....	1: .13 :19.7	1: .21 :21.7	1: .21 : 25.1	-----
Stinson.....	1: .14 :15.5	1: .14 :16.2	1: .30 : 29.4	-----
Sweet Romanite.....	1: .55 :75.2	1: .33 :38.4	1: .69 : 81.6	-----
Terry.....	1: .14 :13.4	1: .16 :16.2	1: .18 : 18.1	-----
Townsend.....	1: .15 :12.3	1: .185:22.6	1: .32 : 39.3	-----
Trader.....	1: .21 :12.3	1: .39 :25.5	1: .14 : 12.4	-----
Trumbull.....	1: .87 :51.2	1: .77 :58.5	1: .94 : 74.3	-----
Utter.....	1: .17 :16.3	1: .18 :20.7	1: .36 : 28.0	-----
Wealthy.....	1: .12 :12.0	1: .13 :16.3	1: .17 : 13.7	-----
White Doctor.....	1: .14 :12.5	1: .16 :22.4	1: .14 : 13.0	-----
White Pearmain.....	1: .167:22.4	1: .16 :23.4	1: .177: 19.3	-----
White Pippin.....	1: .13 :21.1	1: .21 :24.2	1: .16 : 16.9	-----
Willow.....	1: .11 :12.9	1: .16 :19.4	1: .18 : 17.8	-----
Wilson June.....	1: .20 :15.3	1: .25 :21.1	1: .22 : 24.1	-----
Wolseley.....	1: .14 :13.7	1: .13 :15.3	1: .097: 11.1	-----
Winter Smokehouse.....	1: .31 :31.5	1: .209:21	1: .22 : 18.6	-----
Yellow Siberian.....	1: .38 :10.8	1: .34 :11.09	1: .63 : 18.5	-----
York Imperial.....	1: .16 :25.0	1: .16 :26.7	1: .11 : 20.7	-----

In Table 7 the ratios of acid to total astringency and sugar have been tabulated for all varieties which fruited annually in the three years 1922-1924 or in the four years 1922 to 1925. In stating these ratios titratable acidity is taken as unity, and the figures for total astringency and total acidity are quotients obtained by dividing the percentages of each by the titratable acidity. Since in the case of any given variety for a given year the acidity for the year is always taken as unity, and its amount varies from year to year, the ratios for successive years express only the relations existing between these constituents and do not tell anything about the absolute amounts of any of them. This is only another way of saying that the ratio for any given variety and year gives a collective expression of the character of the sample as a whole. In so far as such an expression can be derived from the results of chemical analysis, it states the effect which such a mixture would produce upon the organs of taste. Such effects are not quantitative but comparative and center around the acid content.

An example or two will make the idea clear. The ratios for Baker indicate at a glance that the 1923 and 1925 juices were relatively more astringent and sweeter, and the 1924 juice more astringent, than that of 1922. The ratios for Nero indicate that the 1924 and 1925 juices were very nearly identical, but were more astringent and less acid than that of 1922, with 1923 intermediate in astringency. Akin is quite constant in its acid-astringency ratio, but the 1923 and 1925 juices, which were nearly identical, were relatively much sweeter than those of 1922 and 1924. The juice of Bughorn was closely similar in the first three years, while the juice of 1925 was decidedly more astringent and sweeter.



Since sugars, acidity, and the two classes of astringent materials are variables which fluctuate from year to year with a considerable degree of independence, it is to be expected that the ratios for successive years will show a fairly wide range. This is the case. Close ratios, such as are seen in Arnold, Baldwin, Brackett, Rome Beauty, Yopp, or Westfield, are rare, since they can occur only when the variety has very nearly the same composition from year to year or when all three constituents vary together in the same direction and to nearly the same degree. On the other hand, very wide ratios, such as occur in Jefferis, Martin, Sweet Orange, Algerienne, Victoria Sweet, Jersey Sweet, Springdale, and Red Siberian, may have any one of several explanations. If the ratios are in fairly close agreement except for one year, which has a widely different ratio, as in Springdale, possibility of error in sampling or in analysis is indicated. If the ratios fluctuate widely from year to year, it may indicate that the variety is far out of its natural environment and is especially responsive to variations in seasonal conditions. This may be the case in Sweet Orange, Martin, Alg  rienne, Victoria Sweet, and Jersey Sweet.

If the primary purpose of the acid-astringency-sugar ratio be kept clearly in mind and is considered as giving a conception not of quantitative chemical composition but of the collective effect of a juice upon the palate, the data presented in Table 7 has practical value as well as scientific interest.

#### ASTRINGENCY-SUGAR RATIO

It became evident early in this work that the juices of the group of apples subjected to analysis varied as a whole in their astringency to taste from year to year; that is to say, the juices of large numbers of varieties were apparently more astringent in one year than in another. The tabulation of the results for total astringency in Table 4 has shown that there was no general massing of large numbers of maximum or minimum values for total astringency content in any year, hence the observed differences to taste were not due to differences in absolute amounts of astringent substances present. Further examination of the data indicates that total astringency is the sum of two independently fluctuating variables, neither of which is directly affected by seasonal conditions. The absolute amounts of materials collectively termed "total astringents" therefore can not be directly influenced by seasonal conditions. The ratio borne by astringent materials to sugars present, which may be termed the "relative astringency" of the juice, does not depend upon the absolute amount of astringent materials present, but upon the ratio borne by this quantity to the total sugar present. This ratio will be affected by variation in amount of either component from year to year. It has been shown that the total sugar content of the crop displays definite mass tendencies toward high or low values from year to year, and examination of the data should indicate whether the annual variations in sugar content result in definite mass variations in the astringency-sugar ratio from year to year.

In order to obtain information upon this point, astringency-sugar ratios have been calculated for all the varieties for which data upon three or four successive crops were available. The method employed is that of division of the total sugar content of the variety in each year by its total astringent content. This gives a series of numbers

which express the relative astringency of the juice of the variety in the several years, the smallest quotient indicating highest content of astringents with relation to sugar. The results of application of this method of treatment to the available data are summarized in Table 8. In the group of 81 varieties analyzed in the four years 1922-1925, inclusive, 1924 had a much larger number showing maximum relative astringency than any other year, followed in order by 1922, 1925, and 1923. In the 77 varieties analyzed only in 1922-1924, 1922 and 1924 have 30 and 29 varieties showing maximum relative astringency, respectively, while 1923 has 18 only. Combination of the results for the two groups indicates that 1923 was decidedly lower in relative astringency than any other year. The year 1924 would appear to rank highest, rather closely followed by 1922. The position of 1925 is rather indefinite, the 4-year group has a large number of varieties showing next to maximum astringency in that year, but it probably ranks slightly below 1922. The order of the four years with respect to relative astringency is therefore 1924, 1922, 1925, and 1923.

TABLE 8.—Comparative ranking of apple crop, 1922-1925, with respect to relative astringency content

Year	Number of years compared	Number of varieties	Number of varieties ranking as specified in relative astringency			
			First	Second	Third	Fourth
1922.....	4	81	23	21	17	20
	3	77	30	19	28	-----
1923.....	4	81	7	17	31	26
	3	77	18	31	28	-----
1924.....	4	81	33	14	15	19
	3	77	29	27	21	-----
1925.....	4	81	18	30	17	16

The ranking of the various years with respect to relative astringency does not follow that found for total astringency, for an obvious reason. Relative astringency depends for its position in any year upon two factors, the absolute amount of astringent materials and the absolute amount of sugar present. Any factor which depresses sugar content thereby increases the relative astringency; any factor which increases sugar content acts in the opposite direction upon relative astringency. Any factor increasing or decreasing the absolute amount of any astringent material will of course affect the relative total in the same direction. The year 1924 was the year of the series marked by minimum sugar content and next to minimum acid content. It had a large percentage of maximum nontannins. The relative astringency is high because sugars are low and nontannins high. In 1923, the year of maximum sugar and next to maximum acidity, true tannins were higher than in any other year of the series, but relative astringency is low despite this fact. The intermediate results tend to follow the nontannins. It is clear that seasonal conditions which are favorable to the development of high sugar content tend to depress relative astringency to a low level, and,

conversely, seasonal conditions which depress the storage of carbohydrates in the fruit favor the development of a high level of relative astringency. Years of less pronounced seasonal conditions are intermediate in their results.

This differs in detail but not in general effect from the situation found in grapes. In a large group of grape varieties, maximum sugar content was associated in a majority of cases with minimum total astringency, while minimum sugar content was correlated with maximum total astringency (11). In most varieties of grapes true tannins make up only a small portion of the total astringency, so that the total astringency follows the nontannins irrespective of the independent variation of the true tannins. In apples true tannins make up so large a portion of the total astringent substances that their independent fluctuations affect total astringency, and consequently its absolute amount does not rigidly follow the astringent nontannins. Nevertheless these substances play the dominant rôle in determining relative astringency; that is, in determining the relation of the sum total of astringent substances to total sugars and titratable acidity.

#### CLIMATIC CONDITIONS AT WASHINGTON, D. C., 1918-1925

The location of the apple variety collection of the Office of Horticulture at the Arlington Experiment Farm, within  $2\frac{1}{2}$  miles of the Washington station of the United States Weather Bureau, makes the detailed records of that station available for the purposes of this study. The data presented in the Monthly Meteorological Summary, Form 1030, for Washington (31), and in Climatological Data, Virginia Section, for the years 1917-1925 (32), afford material for a rather complete statement of the seasonal conditions under which the successive crops were grown, and consequently make possible somewhat detailed comparisons of the various years of the period with one another and with the "normal" or 50-year average in so far as certain factors are concerned. These factors are rainfall, sunshine, and temperature, for each of which a daily record is available.

It is of course obvious that comparisons of year with year which are limited to consideration of the factors just named can present only broad outline sketches of the years concerned. For any attempt at quantitative estimation of the conditions under which plants are living in the open, continuous records of soil and atmospheric moisture, wind movement, soil temperature, solar intensity, and rate of evaporation must be available. By reason of their nature these factors must be measured in the immediate vicinity of the plants studied. No such measurements have been made in the present work. While most of these unmeasured factors are determined as regards their nature and amount by the factors of rainfall, sunshine, and temperature, this is not the case for all of them. The measurements of conditions are therefore incomplete, and any comparisons between years are necessarily qualitative, not quantitative, in character.

In the discussion which follows, the data upon temperature, sunshine, and precipitation for the years 1918 to 1925 are first presented as summary statements for the various years, each accompa-

nied by a graph of conventional type in which the data for the calendar year are compared with the values for the "normal" or 50-year average. (See figs. 1-8.) In a subsequent section the data have been rearranged in such a way as to bring the results into more direct relation to the activities of plants by replacing the division into calendar years by a division into crop years beginning October 1 and ending September 30. The data upon each of the three factors—temperature, sunshine, and rainfall—for the entire period are assembled in graphs (fig. 9, rainfall; fig. 10, sunshine; fig. 11, temperature), to facilitate comparison of individual years with one another and with the normal with respect to that factor. In another graph (fig. 12) all the data for the three factors are brought together in summary. Incidental reference to the graphs presenting the data on this basis will be made in discussing the records for individual years, but a detailed statement of the methods adopted in the rearrangement and of the reasons therefor will be postponed until comparison of the various crop years is begun.

Climatological data for the years 1918 and 1919 are presented as giving an idea of conditions preceding the experimental period.

#### CLIMATIC CONDITIONS IN 1918

The winter of 1917-18 was characterized by unusual cold in November, December, and January, the mean temperatures for the three months falling below the normal by 2.2°, 8.2°, and 9.3° F., respectively. February and March were warmer than usual, the daily excess in March amounting to 6.3° and being associated with precipitation and sunshine considerably greater than normal. Clear weather and temperatures below freezing in the last week of March checked plant growth and prevented injury by killing frost on April 6. April was normal in temperature with more than twice the normal precipitation. May was 5.3° warmer, June and July somewhat cooler than the average. August and October were dry and hot, while September was 4° subnormal. The year ended with an accumulated excess of temperature of 321°, the period March 1 to September 30 having 210° of the total. The frostless period extended from April 16 to October 23.

The percentage of sunshine received in February, March, May, and November was considerably above normal, March having 74 per cent of the total possible sunshine as compared with the normal of 51 per cent. May and December had subnormal sunshine combined with above-normal precipitation, while October combined low sunshine with high temperature and little more than one-fourth the normal rainfall. June to September, inclusive, very closely approximated the normal. The total hours of sunshine for the year were 2,679.2 as against a normal of 2,581.4; for the period March 1 to October 1 the total was 1,853.2 hours, an excess of 102.3 hours over the normal average for the period.

The rainfall for the year was materially below normal. November and December of 1917 were dry, having only 2 inches of precipitation, while February had less than one-fourth the normal. Excesses in October and January failed to bring the total for the period October 1 to normal by 3.95 inches. March and April together had 11.62 inches instead of the normal 7.10, but the whole period May to November, inclusive, was one of subnormal rainfall. From March 1

to October 1 the total received was 23.49 inches instead of the normal 27.75. There was consequently a shortage of 8.26 inches in precipitation between October 1, 1917, and October 1, 1918—35.24 inches as against the normal 43.50—and the concentration of nearly one-third of this total in March and April, with persistent drought through the remainder of the season, undoubtedly operated to produce a greater deficiency of soil water toward the end of the growing

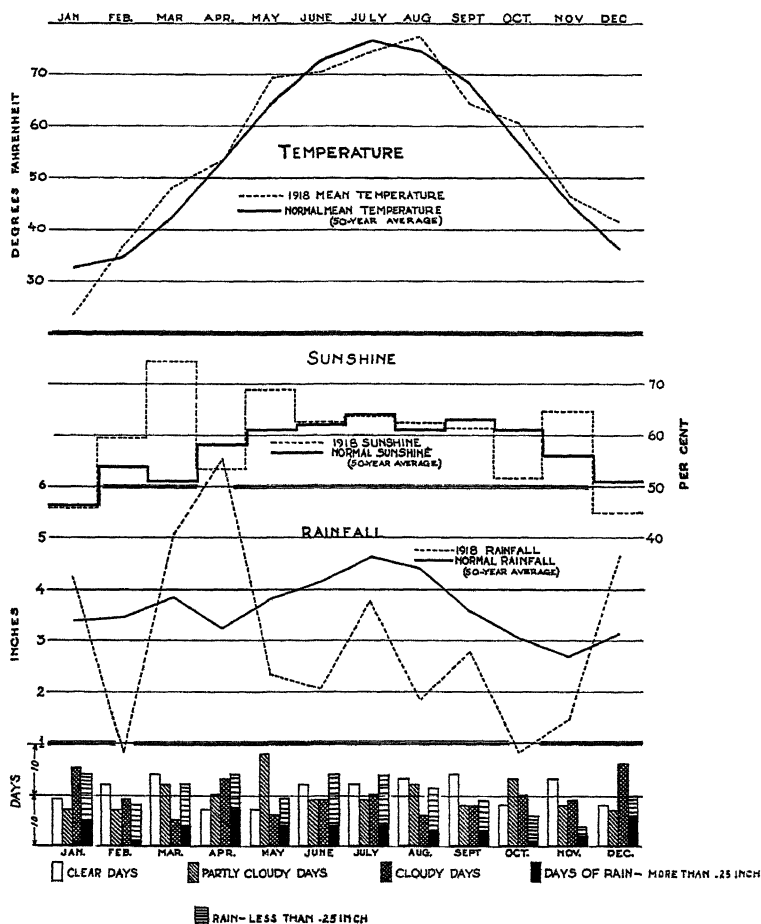


FIG. 1.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1918, with 50-year average for comparison

season than the figures, considered without reference to distribution, would indicate. The subnormal temperatures during June, July, and September operated to reduce the rate of water loss by evaporation during these months, but the shortage was nevertheless large enough to be significant.

The climatic data for 1918, with 50-year average for comparison, are shown graphically in Figure 1.

## CLIMATIC CONDITIONS IN 1919

It is immediately apparent from inspection of Figure 2 that 1919 was a year of consistently above-normal mean temperature. In only two months, August and December, were the averages below normal, and in August the deficiency was less than a degree. The winter months were mild and warm, December and January especially so, and only 3.3 inches of snow fell during the entire winter. While

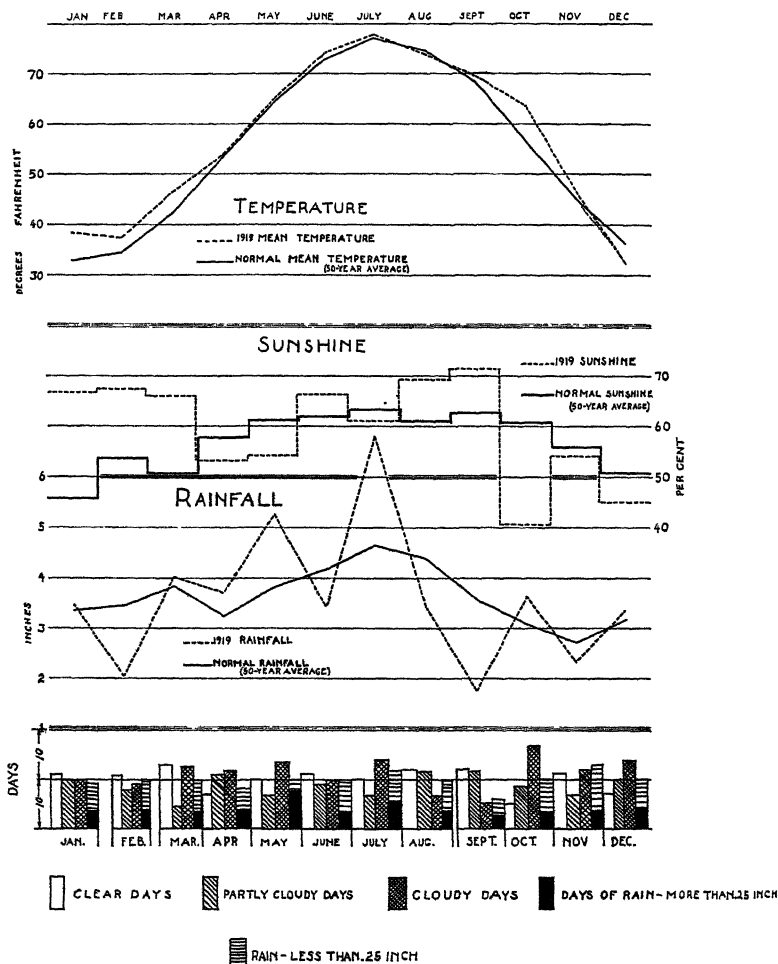


FIG. 2.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1919, with 50-year average for comparison

March had a mean temperature of 4.4° F. above normal, minimum temperatures during both March and April were low enough to prevent premature development of fruit buds, so that very little damage was done by a succession of killing frosts on April 1, 2, 3, and 25 and 26. The departures from normal of the summer months were not large, so that while the calendar year had an excess of temperature of 616° only 226° of this accumulated during the growing

season (March 1 to September 30). The frostless season extended from April 26 to November 10, or three weeks later than the average date.

The percentages of sunshine received in the first three months of the year were exceptionally high, but April, May, and July were slightly subnormal. August and September had considerably more than the average. The total for the calendar year was 2,678.3 hours, as compared with the normal average of 2,581.4. For the period March 1 to October 1 the total was 1,835.8, an excess of 89.5 hours over the normal average of 1,749.9 for this period. The excess largely occurred in March, at the outset of the growing period, and in August and September, when the development of the crop was well toward completion. The rainfall of the year was unusual in that it closely approximated the normal in amount and distribution. There was a deficiency of 2.29 inches for the period October 1, 1918, to March 1, 1919, but for the growing season (March 1 to October 1) there was a total of 28.43 inches precipitation, or 0.68 inch more than normal.

#### CLIMATIC CONDITIONS IN 1920

In 1920 (fig. 3), with the exception of March, which had a mean temperature  $3.4^{\circ}$  F. in excess of the normal, the monthly temperatures from January to the end of July were all subnormal by  $0.5^{\circ}$  to  $4.2^{\circ}$ , May being notably cold. From August onward to the end of the year the monthly means were above normal by  $0.4^{\circ}$  to  $4.5^{\circ}$ . In consequence, an accumulated deficiency of temperature of  $315^{\circ}$  at the end of July was reduced to  $23^{\circ}$  at the end of the year. For the period March 1 to October 1 there was an accumulated deficiency of  $104^{\circ}$ . December, 1919, and January and February, 1920, were colder than normal, and there was a total of 16.4 inches of snow during the winter. The generally backward condition of vegetation prevented injury by the last frosts of spring, which occurred on April 10, 11, and 14. The first killing frost of autumn occurred on October 30, a week later than usual.

The amount of sunshine during the calendar year was only 32 hours under the normal, but distribution was decidedly abnormal. February, November, and December had large deficiencies, August somewhat less, while March, September, and especially October had excesses. Deficiencies in April and May were about equal in amount to the excesses in June and July. The period March 1 to October 1 had 30 hours more than the normal, nearly all of this being due to the excess in March at the outset of the growing season.

The rainfall for the calendar year was 3.09 inches below the normal. The deficiencies were due to shortages in January, March, May, and October which were not equalled by the excesses in April, June, July, August, and November. The shortage was at no time serious enough to be significant. (See fig. 9.) For the winter period, October 1 to March 1, it totaled 15.04 inches, only 0.71 inch less than normal; from March 1 to July 1 it amounted to 13.30 inches, or 1.80 less than normal; while from July to October it totaled 13.28 inches, or 0.64 inch more than normal. The shortage for the period October 1 to September 30 was consequently only 1.87 inch. The crop year therefore more nearly approached the normal in amount and distribution of both summer and winter rainfall than was the case in any other year of the period.

## CLIMATIC CONDITIONS IN 1921

The year 1921 departed materially from normal in all respects. (Fig. 4.) The fall months of 1920 were warm, frost being delayed 10 days later than usual, and January and February were  $3.7^{\circ}$  and  $4.5^{\circ}$  F., respectively, above the normal. While there was 16.4 inches of snow during the winter, it at no time remained on the ground more than three or four days. March and April were the warmest on record in Virginia since the establishment of systematic records (32). The

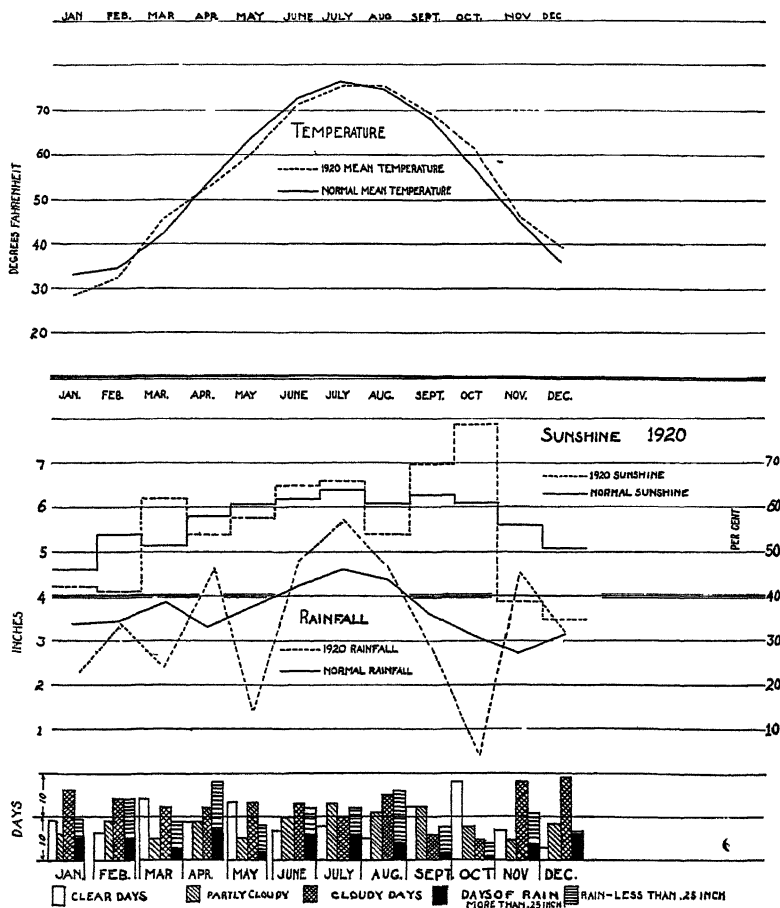


FIG. 3.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1920, with 50-year average for comparison

minimum temperature recorded at Washington during March,  $44.3^{\circ}$ , is  $2.2^{\circ}$  above the normal mean for the month, while the mean was  $13.4^{\circ}$  above normal. The mean for April was  $6^{\circ}$  above normal. In consequence, growth was resumed very early, and was so far advanced that a cold wave accompanied by temperatures of  $26^{\circ}$  and  $27^{\circ}$  on March 29 and 30 did considerable damage, while killing frosts on April 2 and 11 were still more injurious, killing all but a very small percentage of fruit buds on most of the early-blooming varieties and causing considerable injury to the later varieties.



The year as a whole was hot and dry, only May and August having mean temperatures below the average. The accumulated excess of temperature for the year equaled 1,127° or an average daily excess of 3.1°. The year is characterized as "the warmest and driest year in Virginia since the beginning of State-wide records in 1891," and, further, as "one of the most unfavorable for agricultural interests ever experienced in Virginia" (32) and the data for the Washington

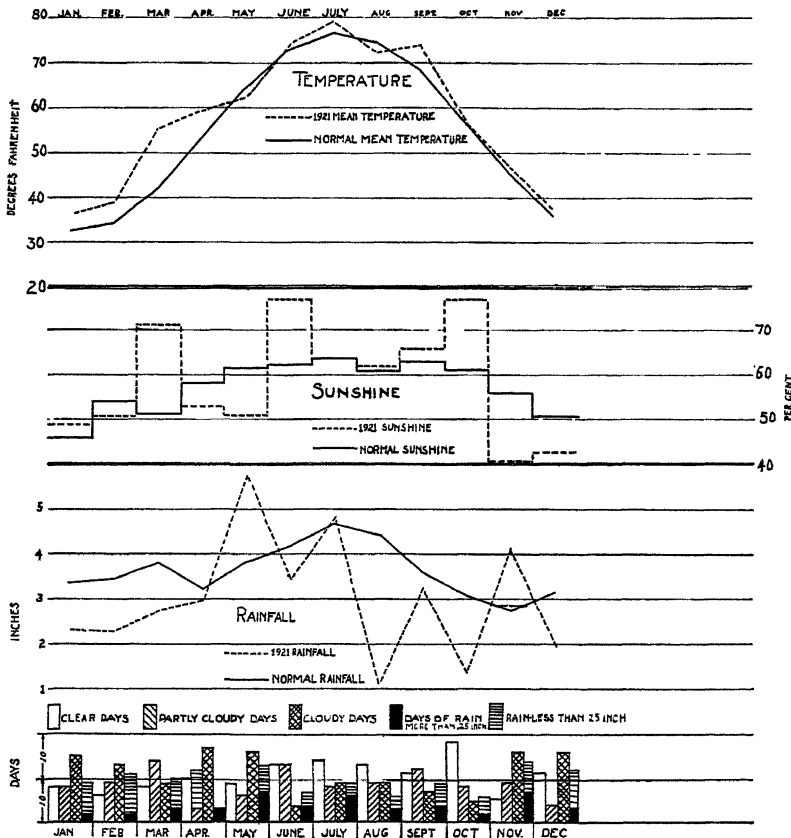


FIG. 4.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1921, with 50-year average for comparison

station indicate that these statements may be applied without modification to the local conditions.

In so far as temperatures for the growing season are concerned, the period March 1 to October 1 accumulated an excess of 792°, or 3.7° per day. Much the larger portion of this total accumulated in April and the remainder in September, the net increase from May to the end of August being but 11°.

The distribution of sunshine for the year was abnormal. (See figs. 4 and 10.) The total amount was 2,666 hours, or 84.6 hours more than the average. Three months—March, June, and October—had notable excesses; April, May, and the last two months of the year had considerable deficiencies. More than the normal share of the

total was received between March 1 and the end of September, as this period had 1,846.7 hours instead of the normal 1,749.9.

Rainfall for the calendar year was deficient by 7.37 inches, the total being 36.13 inches. The first four months of the year were considerably deficient, August and October notably so. May had the largest precipitation for the year, but this exceeded the average by less than 2 inches. Considered on the basis of the crop year, October to February, inclusive, had 12.65 inches of precipitation, so that the

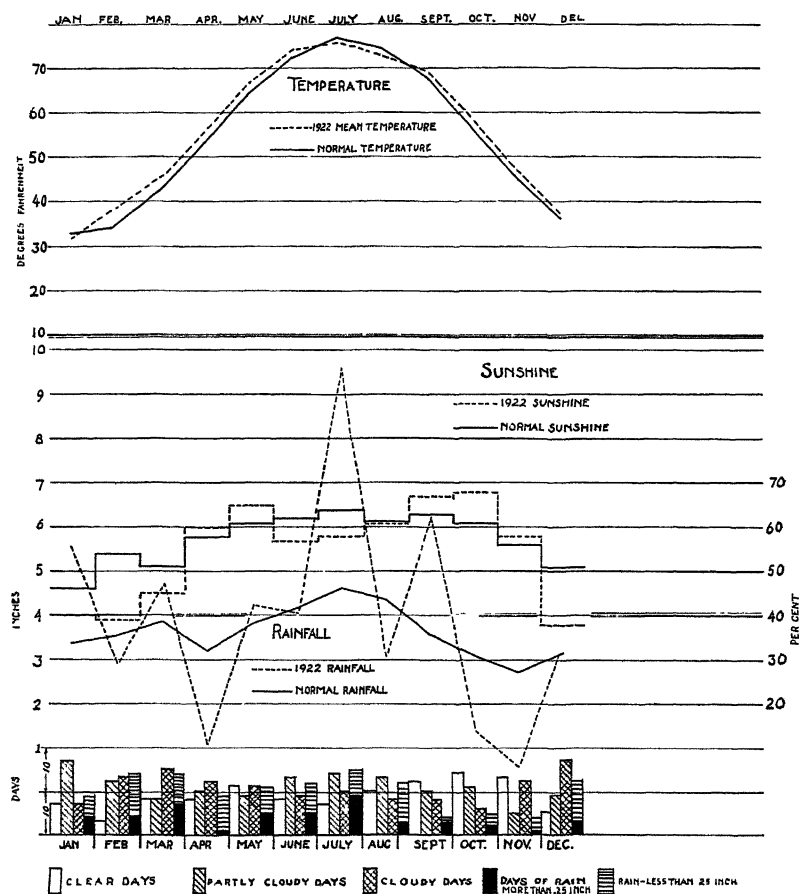


Fig. 5.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1922, with 50-year average for comparison

growing season began with a shortage of 3.1 inches. The period March 1 to June 30 had 14.91 inches, only 0.2 inch less than normal, but the remainder of the growing season had only 9.18 inches, a shortage of 3.46. The actual shortage of available water was undoubtedly somewhat greater than the absolute deficiency of 6.77 inches for the year considered alone would indicate, since high temperatures and above-normal sunshine operated to increase surface evaporation from the soil and transpirational water loss from plants throughout the growing season.

## CLIMATIC CONDITIONS IN 1922

The year 1922 (fig. 5) averaged  $1.5^{\circ}$  F. warmer than normal. January, July, and August were slightly below the average, but all the other months ranged above it by amounts varying from  $1.8^{\circ}$  to  $4.2^{\circ}$ . The temperatures of the late months of 1921 were all above the normal, and the winter was mild. Snow fell in December, January, February, and March, but the unusual total of 44.5 inches was due to the fall of 26 inches on January 27 and 28. With the exception of this heavy fall, some of which remained on the ground until late in February, the ground was bare except for two to four days at a time throughout the winter. The warm winter and spring resulted in early awakening of fruit trees, with the result that apple trees were rather generally coming into full bloom when a series of frosts occurred on April 21 to 29. The damage ranged from 25 to 75 per cent in commercial orchards. Some early blooming varieties in the variety collection escaped with only negligible damage. The growing period, March to September, was slightly less warm than the year as a whole, the daily excess of temperature being  $1.34^{\circ}$ .

Sunshine was subnormal in the first three months of the year, in slight excess in April and May, below average in June and July, and again in excess in September and October. The total for the year was 2,477.2 hours, or 104.2 hours less than normal; but the period March 1 to September 30 had 1,714.1 hours, or only 35.7 less than the average for these months.

Rainfall was 3.46 inches in excess of normal for the calendar year. July was the wettest since 1905, having 9.59 inches, while the 0.55 inch recorded for November is the smallest precipitation occurring in that month since 1871. Considered on the basis of the crop year, the precipitation from October, 1921, to March was 15.89 inches, or 0.12 more than normal; that for March to July 14.16 inches, or 0.95 less than normal; that for July to October 18.94, or 6.3 more than normal. The total for the crop year was 48.99 inches, or 5.49 inches more than the 50-year average.

The crop year was consequently slightly deficient in sunshine, had a material excess of heat well distributed through the season, and a large excess of precipitation, concentrated in the last half of the growing season.

## CLIMATIC CONDITIONS IN 1923

Mean temperatures for 1923 departed materially from the average only in January, March, June, and December. (Fig. 6.) In the remaining months the means were within  $1^{\circ}$  F. or less of the average, except in September and October, which were, respectively,  $1.5^{\circ}$  above and  $1.3^{\circ}$  below normal. The mean for December was the highest ever recorded by the Washington station for the month, with the exception of the year 1886. While March and April were above normal in their mean temperatures, early resumption of growth was prevented by minimum temperatures of  $21^{\circ}$  and  $19^{\circ}$  F., respectively, on March 19 and 20, and of  $18^{\circ}$  and  $15^{\circ}$  on March 29 and April 1, with minima near or below freezing preceding and following these dates.<sup>4</sup> In consequence, heavy frosts on April 7, 9, and 10, with a light sleet storm on the 14th and 15th, did no damage to apples.

<sup>4</sup> The figures given are taken from the Monthly Meteorological Summary, Form 1030, for Washington, March, 1923 (31).

The growing season was warmer than normal by an average of  $0.85^{\circ}$  per day, the excesses of March, April, and June considerably exceeding the deficiencies of July and August.

The period March 1 to June 30 was remarkable for the large excess of sunshine in every month. The total of 1,185.1 hours is in excess of the normal by 218 hours. That the growth and development of crops was appreciably accelerated by the increased photosynthetic activity thus made possible is evident when it is considered

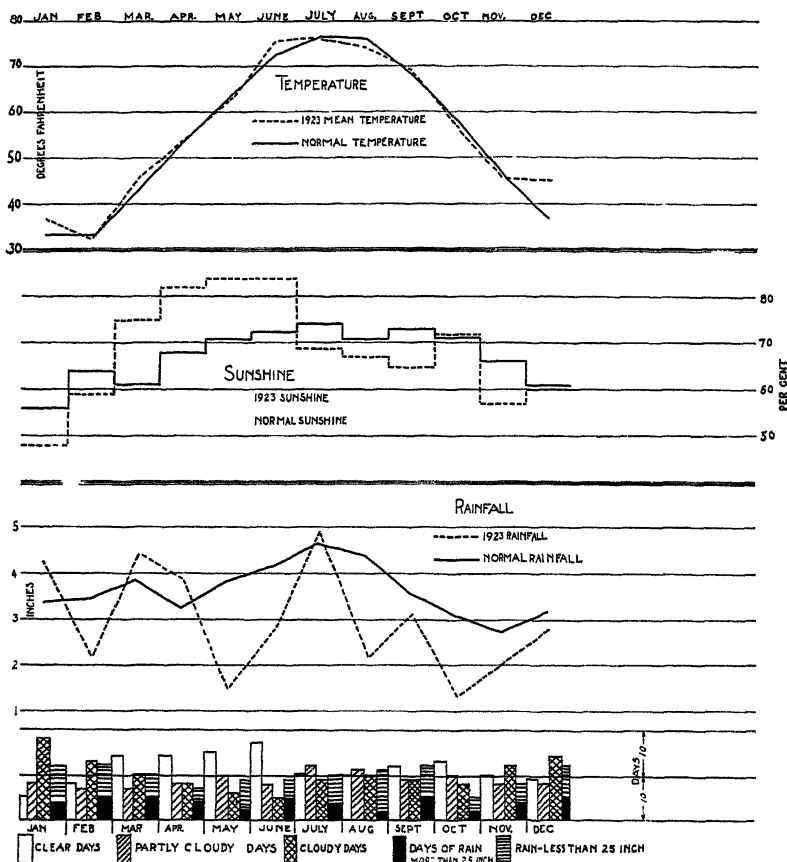


FIG. 6.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1923, with 50-year average for comparison

that the effect is that of adding 17 days, each with 13 hours' continuous sunshine, to the effective working time of plants during the period. For the remainder of the growing season sunlight was consistently subnormal, July, August, and September having deficiencies totaling 66.6 hours. The growing season had a total of 1,901.4 hours, or 151.5 more than normal, but its distribution over the growing period was such as to accelerate development in the first half and to retard it in the last half of the season.

The precipitation for the calendar year was 7.9 inches below the normal. Snowfall was two-thirds the average. Only four

months had rainfall exceeding the normal, and the excesses were in all cases small. Deficiencies in October and November of 1922 resulted in a shortage of 8.66 inches for the crop year ending September 30. The winter period had a deficiency of 3.88 inches, March 1 to July 1 a deficiency of 2.40 inches, and the remainder of the growing season a further deficiency of 2.38 inches. There was consequently a soil-water deficit at the outset of the crop season which was never even momentarily made up, but which steadily

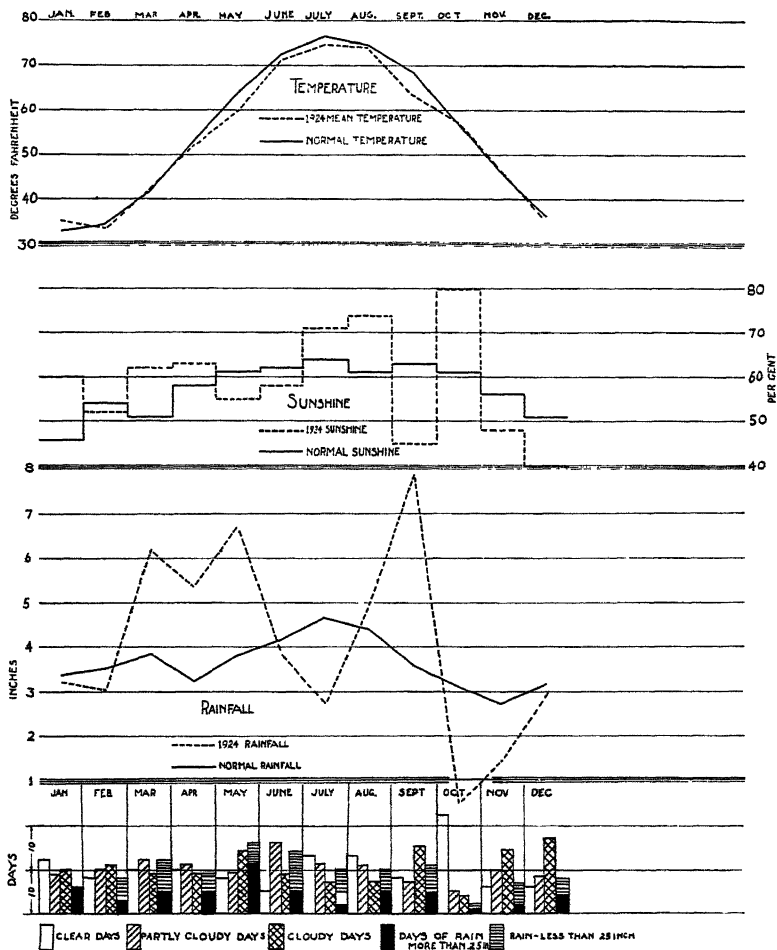


FIG. 7.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1924, with 50-year average for comparison

increased through the entire period of growth and maturity. Stated in another way, the soil received in the 12 months ending October 1 only the amount of precipitation normally received by the end of July.

#### CLIMATIC CONDITIONS IN 1924

The year 1924 was cold and wet, yet had slightly more than the normal amount of sunlight. (Fig. 7; see also figs. 9 and 10.) The entire spring and summer were consistently subnormal in tempera-

ture, an accumulated excess of  $17^{\circ}$  F. at the end of February being converted into a deficiency of  $341^{\circ}$  at the end of September, or an average daily deficiency for the season of  $1.67^{\circ}$ . May was exceptionally cool, having a daily deficiency of  $3.7^{\circ}$  associated with high rainfall and subnormal sunlight, but was exceeded in all these respects by September, which had a mean temperature of  $3.9^{\circ}$  below normal, only 71 per cent of the normal hours of sunshine, and more than twice the average precipitation. Considered upon either the calendar-year or the crop-year basis, 1924 was by a considerable margin the coldest season of the period covered by these studies.

The sunshine for the year exceeded the normal by 68.7 hours. Its distribution was such that the months, March to September, inclusive, received 37.4 hours more than the normal, a little more than half of the excess being received prior to July 1. The fluctuations from month to month can be better appreciated from the graphic record (figs. 7 and 10) than from a statistical statement.

Rainfall was in considerable excess for the year, especially in the spring months. The heavy precipitation in March canceled a deficiency of 3.29 inches which had resulted from subnormal rainfall in each of the five months from October to February, inclusive. The four months ending June 30 had 22.18 inches of rain, an excess of 7.07 inches, while the three months ending September 30 had 3 inches more than the normal. The growing period of seven months thus had a total of 37.82 inches, or an excess of 10.07 inches. This amount exceeds the totals received in the 12 months ending September 30 in the years 1918, 1921, 1923, and 1925.

#### CLIMATIC CONDITIONS IN 1925

The year 1925 showed material departures from normality in rainfall, sunshine, and temperature. (Fig. 8.) February was  $7.8^{\circ}$  warmer than usual, a record previously equaled only in 1890 and 1909. March and April had averages  $3.8^{\circ}$  and  $3.6^{\circ}$  F., respectively, in excess of normal, May was  $3^{\circ}$  subnormal, and June was the hottest month of the year, with  $5.2^{\circ}$  daily excess. September had a daily excess of  $4.7^{\circ}$ , while October was cold, with a daily deficiency of  $5.3^{\circ}$ . An accumulated excess of temperature of  $204^{\circ}$  at the end of February increased to  $491^{\circ}$  at the end of June and to  $569^{\circ}$  at the end of September. The four months March to June, inclusive, had an average daily excess of  $2.35^{\circ}$ , while the remaining three months of the growing season had an average of  $0.86^{\circ}$  per day.

The first six months of the year each had more than the average amount of sunshine, the excess from March 1 to the end of June totaling 151.8 hours. This is the equivalent of the addition to the period of 12 days each having 12.6 hours of uninterrupted sunshine. July had a deficiency which was not quite balanced by the excess of August, and the deficiency in September reduced the total for the three months to 720 hours, which is 63 hours less than normal for the period. (See figs. 10 and 12.)

The rainfall for the crop year ending October 1 was the smallest for any year of the period under study. From October 1, 1924, to March 1, 1925, the total, 10.31 inches, is less than two-thirds the normal amount for this period. It must be noted, however, that torrential rains occurred over most of Maryland and Virginia on September 29 and 30, the total precipitation for the two days at Washington

equaling 5.44 inches. While the character of this downpour resulted in maximum run-off, it undoubtedly contributed in some degree to the stored soil water, with the result that the shortage on March 1, 1925, was actually somewhat less than the 5.44 inches indicated by comparison of the figures for precipitation between October 1 and April 30 with the normal.

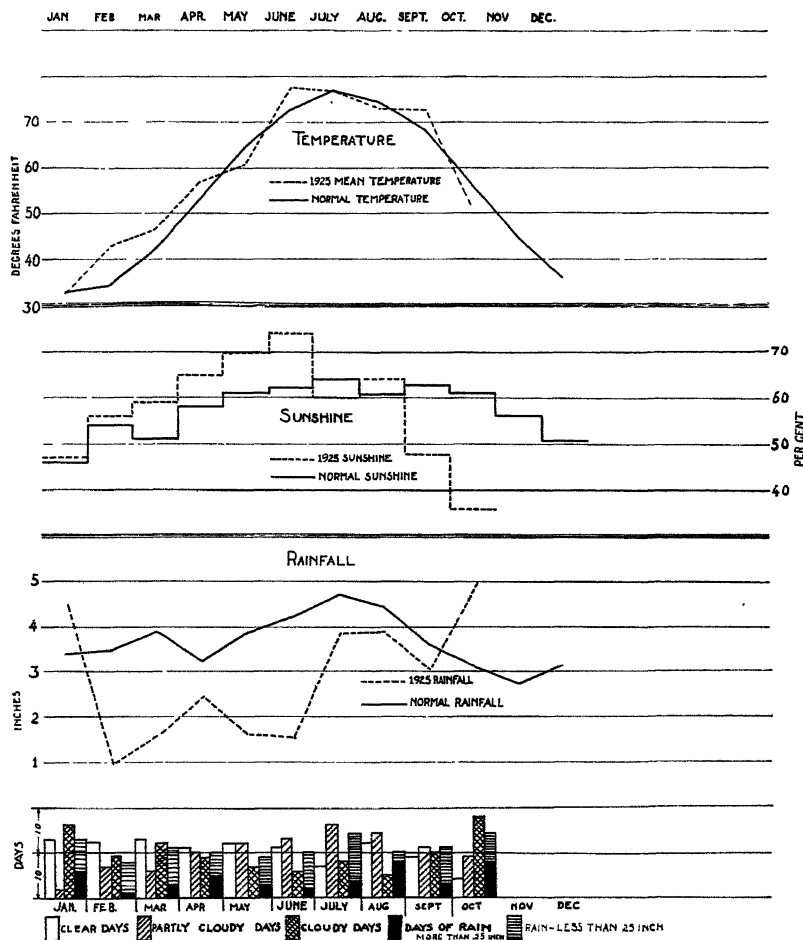


FIG. 8.—Climatic data (monthly mean temperature, sunshine, and rainfall) for Washington, D. C., for 1925, with 50-year average for comparison

The entire growing period was characterized by consistently sub-normal rainfall in every month. The amount received from March 1 to the end of June was only 7.24 inches, not quite 48 per cent of the average. The last three months of the growing period had a deficiency of 1.91 inches. The total for the year ending October 1 was 28.31 inches, a deficit of 15.20 inches, or 34.2 per cent. The growing period had 18 inches, which is less than two-thirds the normal of 27.75 inches and less than one-half the high total of 37.87 inches for this period occurring in 1924.

## CLIMATIC DATA IN RELATION TO CROP PERFORMANCE

In the preceding section the data for rainfall, sunshine, and temperature for each of the calendar years under study have been compared with the normal or 50-year average, and the extent of the departures from normal in amount and distribution of each of these factors has been stated. Such treatment gives a conception of any individual year as a unit in terms of the normal, as colder, drier, or wetter than the average, but it does not permit satisfactory direct comparison of one year with another or permit such a mental grasp of the conditions during a series of years as enables their evaluation in terms of the response of plants. In order to compare the climatic data for the series of years with relation to the production of crops, a rearrangement is necessary. Such a rearrangement has been made in Tables 9 to 12 and in Figures 9 to 12 on the basis of a crop year beginning October 1 and ending September 20.

The employment of the date of the first killing frost as the end of the crop year would involve comparison of periods of varying length. October 1, which in this latitude is very nearly the middle of the apple harvest, appears to fit the case better than any other arbitrarily chosen date could do. It has already been used by Shaw (30) as the end of the growing season. The crop year has been further arbitrarily divided into an inactive or dormant period, beginning October 1 and ending March 1, and a period of active growth, extending from March 1 to September 30. This division, which has also been employed by Shaw (30), has as its justification the fact that while the period of dormancy is far from being one of inactivity, the activities of the plant are not such as can be easily measured. The plant gives no immediate response which can be detected to the variations in intensity of the climatic factors, their effect becoming evident only as they affect the behavior of the tree during the succeeding period of active growth. In the active period the amounts of rainfall, heat, and sunshine received and their distribution in time are directly effective in determining the rate at which the life processes may go on, and their fluctuations from day to day determine the amount of vegetative growth and the quantity and quality of fruit produced.

The selection of March 1 as an arbitrary date for beginning the growing season has much to support it. It is rather well established that for a very wide range of plants a mean daily temperature approximating 40° F. represents a lower limit beyond which appreciable growth does not occur (1, 13, 19, 28), and this rounded figure is generally adopted as the approximate minimum temperature for growth. This study deals with a large number of horticultural varieties which show very considerable differences in the readiness with which they respond visibly to the increasing temperatures of spring. The date at which the daily mean temperature first equals 40° may be employed as a starting point for the active period with such an assemblage of forms, since the purpose of establishing such a point is merely to enable a comparison of like periods of time in various years with one another and with a "normal."



Adopting this conventional figure, it is found from the records of the Washington station<sup>5</sup> that the normal mean daily temperature at that station reaches 38° F. on February 27, 39° on March 3, and 40° on March 7. As the average mean temperatures for March exceeded the normal in every year of this study by 3.1° to 13.4° except in 1924, which was exactly normal, no material error will be involved in fixing upon March 1 as the beginning of the growing season, which will be considered as consisting of the seven months from March to September, inclusive.

For purposes of comparison the growing period is divided into two portions, that prior and that subsequent to July 1. The period March 1 to July 1 is that in which the tree develops its foliage and blossoms, and it includes the critical periods in which the tree may respond to unfavorable conditions by cutting down its load of fruit through dropping. The period subsequent to July 1 begins at a time when the load of fruit on the tree has been determined; the tree does not subsequently respond to unfavorable conditions by dropping its fruits. July 1 is about the time at which the "rest period," indicated by the formation of terminal buds, begins in the apple (21, 14, 17). Gourley (18) found that the annual increase in length of apple twigs in New Hampshire was largely completed by the end of June. Conditions during the period July 1 to October 1 will determine the size, color, keeping quality, and time of maturity of the fruit, the production of fruit buds for the next crop, and the amount of food reserves accumulated in the tree for use in the succeeding year.

It is obvious that the dates chosen in no sense mark the limits of definite phases of the activities of plants. The plant does not pass suddenly from quiescence to activity, or the reverse, like a machine controlled by a switch; the transitions are gradual and merge insensibly one into another. Nor does it do only one thing at a time; the various activities of the plant overlap. Transformations of reserves, extension of the vegetative system, and development of the crop go on concurrently; maturing of the crop, formation of next year's fruit buds, and storage of reserves for next year's activities occur together. The so-called dormant season is a period of chemical activity which bulks large in its effects upon the accomplishment of the tree in the following active period. It is clear, however, that there are periods of the year in which one or another group of activities are dominant and in which climatic conditions will express themselves in the behavior of the plant primarily through the effects produced upon the dominant activity of the period concerned. Considering the period October 1 to February 28 as primarily one of chemical preparation of materials for next season's use, that from March 1 to July 1 as a period of construction in which accumulated reserves and daily income through photosynthesis are expended in growth, and that from July 1 to September 30 as one of completion of the year's undertakings and of building

<sup>5</sup> Monthly Meteorological Summary, Form 1030, for Washington, D. C., March, 1920, and March, 1925 (31). Normal daily mean temperatures are not directly stated, but are arrived at by adding or subtracting the "departures from normal" from the recorded daily means. Departures from normal are not included in the Monthly Summary for Washington prior to February, 1920.

reserves for the next year, climatic conditions in any period will be considered as affecting the dominant activity of that period. On this basis, comparison of a series of years one with another can be made.

A rearrangement of the weather data on the basis of crop years, beginning October 1 and ending September 30, for the period from October 1, 1917, to October 1, 1925, has been made in Tables 9 to 12 and in Figures 9 to 12. The considerations which have governed in

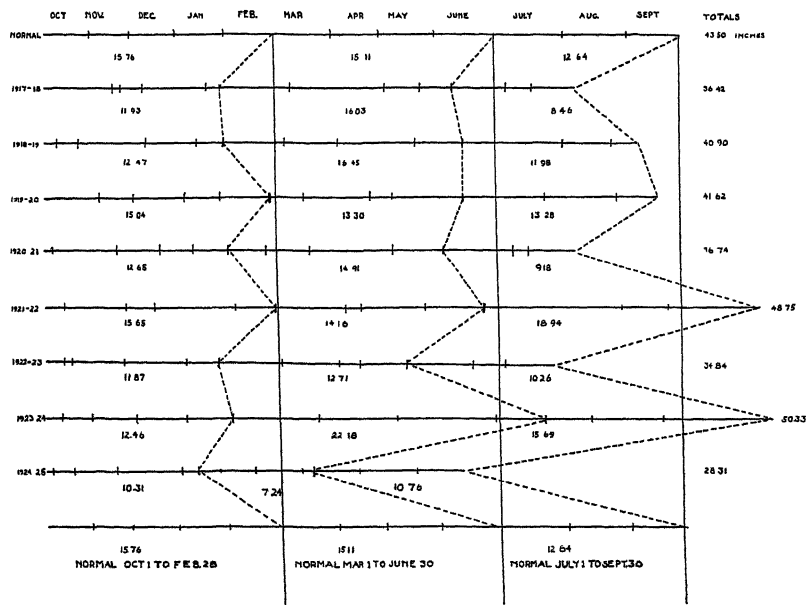


FIG. 9.—Rainfall by months and by periods for the crop year (October 1 to September 30) for the years 1918-1925, with normal 50-year average for comparison. Normal for each period is indicated by vertical lines, broken lines indicate departures from normal for each period in the various years.

the adoption of the methods employed in the rearrangement of the data may be stated at the outset.

In Table 9 the amount of rainfall for each month and the totals for each of the three periods of each crop year, with departures from the normal 50-year average, are stated for each of the crop years. Figure 9 presents the data graphically. Sunshine, which was represented in graphs for the individual years (figs. 1 to 8) as percentages of the total possible sunshine, has been recalculated from the Weather Bureau data as actual hours of sunshine per month and compared with the normal similarly recalculated, with departures from normal, for the same periods as the rainfall (Table 10 and fig. 10).

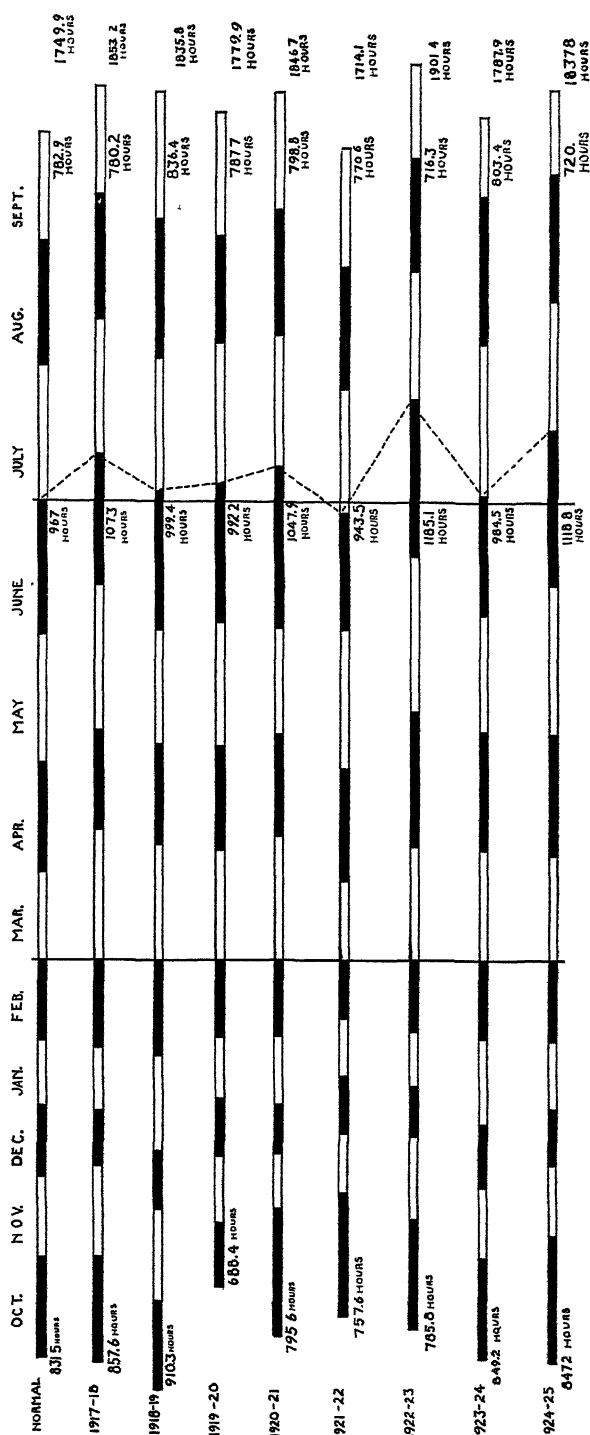


Fig. 10.—Hours of sunshine by months for the years 1918-1925, with normal for comparison. The vertical line representing March 1 is a line of reference, sunshine for October 1 to February 28 being plotted to the left and that for March 1 to September 30 to the right. The broken line connects the totals for the period March 1 to June 30 with normal for the period



Temperature, which has been expressed in the graphs for individual years in the conventional way, by curves showing the departures of monthly mean temperatures from the normal mean, is expressed in Table 11 and Figure 11 on the basis generally adopted in studies of temperature in relation to plant behavior, namely, the summation of temperatures above a base line. While various points on the thermometer scale have been adopted by different workers, 32° F. has been most widely employed as a basis from which to make summations. This seems rather illogical, since it includes in the summation daily heat units well below the minimum for growth. It seems much more rational to adopt the suggestion of Duggar (15) that an approximate growth minimum be taken as a basis and that the difference

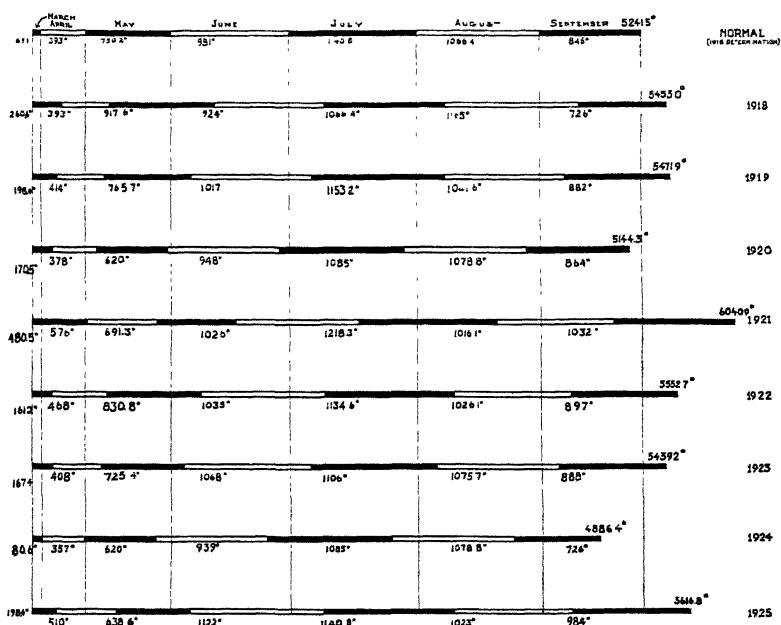


FIG. 11.—Total heat in excess of 40° F. daily received during the months of March to September, inclusive, for the years 1918-1925, with normal for comparison

between this and the daily mean be taken to represent the daily efficiency during the growing season. He suggests 40° F. as the growth minimum. It has been used by McLean (26) and by others, and has been employed by Livingston (24, 25) in his attack upon the problem of integrating temperature values in relation to plant growth. The conventional constant of 40° F. is consequently here employed as the minimum temperature or zero point from which the summations are made.<sup>6</sup> The results are presented in Table 11 and in Figures 11 and 12.

<sup>6</sup> The various totals of heat units above 40° F. given in Table 11 can be converted into heat units above 32° by increasing each by 1,712° (the product of multiplying the 214 days of the growing period by 8, the difference between 32° and 40°) or by a similar method for the shorter periods. Such treatment, of course, does not in any way affect the amounts of the departures from normal given in the table.

TABLE 11.—*Summation of heat units above 40° F. (in degrees Fahrenheit) from March to September inclusive, for the years 1918–1925, with departures from normal year*

Year	Mar. 1 to June 30	Departure from normal	July 1 to Sept. 30	Departure from normal	Total, Mar. 1 to Sept. 30	Departure from normal
Normal.....	2,188.3	0	3,053.2	0	5,241.5	0
1918.....	2,495.0	+306.7	2,958.0	−95.2	5,453.0	+211.5
1919.....	2,395.1	+206.8	3,076.8	+23.6	5,471.9	+230.4
1920.....	2,116.5	−71.8	3,027.8	−25.4	5,144.3	−97.2
1921.....	2,773.8	+585.5	3,267.1	+213.9	6,040.9	+799.4
1922.....	2,495.0	+306.7	3,057.7	+4.5	5,552.7	+311.2
1923.....	2,368.8	+180.5	3,070.4	+17.2	5,439.2	+197.7
1924.....	1,996.6	−191.7	2,889.8	−163.4	4,886.4	−355.1
1925.....	2,469.0	+280.7	3,147.8	+94.6	5,616.8	+375.3

The method employed in arriving at summations of temperatures when short periods are under consideration is to subtract the zero point from the mean temperature of each day and add the remainders. The application of this method to a long series of years is very laborious. A very close approximation to the true values can be arrived at by subtracting the zero value, in this case 40° F., from each of the

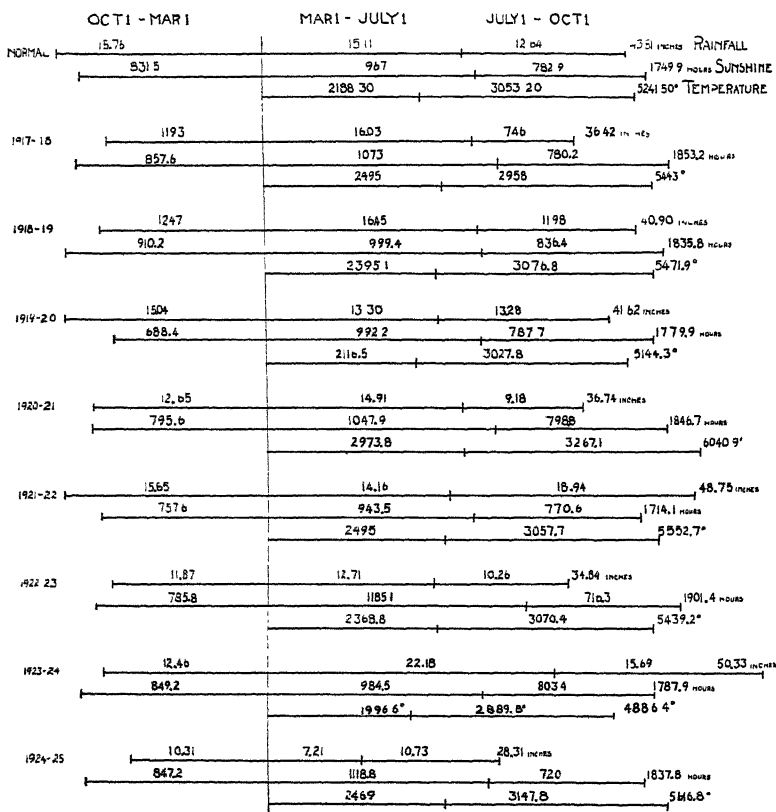


FIG. 12.—Collective summary of climatic data for the years 1918–1925, with normals for comparison. The vertical line representing March 1 is a line of reference, rainfall and sunshine for October 1 to February 28, inclusive, being plotted to the left, and these items with temperature in excess of 40° F. for the period March 1 to September 30, inclusive, to the right

monthly means and multiplying the remainder by the number of days in the month. Values so obtained will differ from the true values only if some of the daily means fall below the constant used as a zero point. Such days are left out in the day-by-day summation, but are included in making up the monthly mean, which they render lower in value. As a matter of fact days with a mean temperature below 40° occur during the growing period in this latitude only in early March and are so infrequent that the error involved in neglecting them is insignificant.

In the case of the period from October 1 to February 28 summations of temperature can not be computed from 40° F. as a basis, since many of the means are below this figure. Some other method must be employed. The ultimate result of any method which can be used is the obtaining of a figure which can be compared with the normal or average, which is everywhere employed as a standard of comparison. The most satisfactory method of obtaining a figure which will give a conception of the character of temperature for the winter period as a whole is to employ the 50-year average as a normal or base line and to sum up the departures, positive or negative, from this normal for each year under consideration. Such a summation, in conjunction with a statement of the amount and character of the departures from normal month by month, is given in Table 12.

No winter injury occurred at any time during the period of the work, and there is no indication that winter temperatures at any time affected the condition or fruitfulness of the trees.

TABLE 12.—*Comparative departures of mean temperatures (in degrees Fahrenheit) from the normal mean temperatures from October to February, inclusive, for the years 1917-1925*

Year	October	November	December	January	February	Total
1917-18.....	-147	-71	-253	-287	+65	-693
1918-19.....	+126	+37	+170	+163	+75	+571
1919-20.....	+206	+55	-109	-128	-53	-29
1920-21.....	+140	+24	+98	+115	+126	+503
1921-22.....	+10	+77	+7	-29	+117	+182
1922-23.....	+63	+79	+33	+107	-79	+203
1923-24.....	-39	-2	+261	+51	-34	+237
1924-25.....	+7	+24	-6	-13	+217	+229

#### COMPARATIVE SUMMARY OF CLIMATIC DATA

Separate comparisons of the data upon the individual climatic factors for the various years of the period under study will facilitate the consideration of these factors taken collectively in an attempt to evaluate them in terms of their effect upon the plant.

##### RAINFALL

It is immediately obvious from inspection of Figures 9, 10, and 11 that of the three climatic factors considered, rainfall is that which displays largest departures from normality both in amount and distribution. When the rainfall records for the crop years (October 1 to September 30) are compared among themselves, several generalizations can be formulated. (See fig. 9.) The crop years 1919 and 1920 are nearest to normal both in total amount of rainfall and in its

distribution through the year. The departures from normal are such as to preclude the possibility that precipitation, considered alone, has been a limiting factor in crop production, and these years may stand as normals so far as this factor alone is concerned. If an excess of 5 to 7 inches for the year, largely concentrated in the growing season, measurably affects the composition and quality of the crop as a whole, 1922 and 1924 should show such effects. If reduction of rainfall for the crop year by 6 to 9 inches affects the crop in a definite manner, 1918, 1921, and 1923 should show such effect, and an intensification of such effect should be evident in 1925. Extremes in water supply during the growing season are presented by 1924 with 37.87 inches and 1925 with 18 inches, and any effects upon crop composition due solely or predominantly to excess or deficiency of precipitation should be apparent when the crops of these years are compared, employing the crop of 1920 as a standard. Summarizing, 1919 and 1920 approach normal very closely; 1918, 1921, and 1923 are years of moderate deficiency; 1925 is one of extreme deficiency; and 1922 and 1924 are years of very considerable excess in precipitation.

#### SUNSHINE

Total hours of sunshine per crop year show a considerably narrower range of variation than does rainfall. This factor has fallen below normal only twice, in 1920 and in 1922, exceeding the average in all the other years. (See fig. 10.) It has materially exceeded the normal for the late autumn and winter months only once, in 1919; it was very close thereto in 1918, 1924, and 1925, and was considerably deficient during this period in 1920 to 1923, inclusive. For the growing season it has been subnormal in one year only, 1922, and that by only 2 per cent of the normal total. In 1920 and 1924 the totals received exceeded the normal by 30 and 37 hours, respectively, or approximately 2 per cent. These differences are certainly too small to be of significance, and 1920, 1922, and 1924 may be considered as normals with respect to this factor. In 1918, 1919, 1921, and 1925 there were excesses ranging from 85 to 103 hours, or 5 to 6 per cent, while 1923 had an excess of 151.5 hours, or 8.5 per cent.

These increases in the working period of the photosynthetic apparatus, amounting as they do to 7 to 12 days of 12 hours uninterrupted sunshine each, are large enough to make recognizable additions to the work accomplished by trees during the season. Whether such increases may be expected to express themselves as increases in vegetative growth and load of fruit or as changes in the composition of the fruit can only be determined by examining the distribution of sunshine over the growing period. The years 1919 and 1925, for example, differed by only 2.6 hours in total sunshine for the season, but 1925 received 1,118.8 hours prior to July 1 as against 999.4 hours for 1919, while 1925 had 720 hours of sun between July 1 and September 30 in contrast with 836.4 hours for 1919. The two years were therefore very dissimilar, since one had a period of development 10 days longer and a period of maturation 10 days shorter than the other, so far as sunshine is concerned. The years 1922 and 1923 are extremes in point of sunshine received, as 1923 received 1,185.1 hours in the period March 1 to July 1, or 241.6 hours more than 1922, but for the period July 1 to September 30, 1923, had the greatest deficiency encountered during the period, 66.6 hours less than the



normal and 54.3 less than 1922. In consequence, 1923 had the largest amount of sunshine in the first half and the smallest amount in the latter half of the season found in the entire period. The results of excesses of 106 hours and 80.9 hours in the first half of the season in 1918 and 1921, followed by amounts close to normal in the second half, will be different in character from the results of 151.8 hours of excess sun in the first half of the 1925 season, followed by a period of maturity having 63.3 hours less than normal sunlight.

These instances serve to emphasize the fact that mere summation of the amount of a climatic factor, unaccompanied by consideration of its distribution over the growing period of the crop, does not give a dependable indication of the probable effect of that factor.

#### HEAT UNITS

There are very considerable variations in the amounts of heat received during the growing season. The years 1921 and 1924 present extremes, 1921 having heat units equaling 123.7 per cent of the total for 1924. (Fig. 11.) With the exception of 1920 and 1924, all the years had summations somewhat above the normal 50-year average. The year 1920 most nearly approached the normal, having a deficiency of 1.8 per cent, while 1924 had a deficiency of 6.7 per cent. The years 1923 and 1918 had excesses of 3.7 and 3.8 per cent, respectively; 1919, 4.3 per cent; 1922, 5.9 per cent; 1925, 7.1 per cent; 1921, 15.25 per cent. If excess or deficiency of heat units received is the dominant factor in determining the character of the crop in any of the years covered by this study, such dominance must manifest itself in the years 1921 and 1924. In distribution of the heat received over the growing season, 1920 and 1923 most nearly approach the normal, the former having a small deficiency, the latter a small excess at the end of each month, throughout the season. If considerable excesses of heat received prior to July 1 with approximately normal amounts thereafter exert recognizable effects, 1922, 1923, and 1918 should show such effects.

In order to form conceptions of the probable effects upon the productiveness of plants of the seasonal conditions of the series of years under study, it is necessary to employ a collective treatment of the data for each year in order to permit comparisons between years. In such a treatment it is indispensable that the conditions of some one year be selected as a standard with which those of the others may be compared. The year 1920 is best suited to serve as such a standard, since it more closely approximates the 50-year average than does any other of the series. The rainfall for the crop year, while falling short of the normal total by 1.89 inches, or 4.34 per cent, was nearer the normal and was also more evenly distributed over the three periods of the year than is the case in any other year. (Figs. 9 and 12.) While the temperatures for the individual winter months depart considerably from the 50-year average, the total departure from the normal for the period October to February was only 29°, which is insignificant. (Table 12.) The heat units received during the growing season were 98.2 per cent of normal, and their distribution over the growing season was exceptionally uniform, May being the only month having much departure. (Fig. 11.) Total sunshine for the year had a deficiency of 113.1 hours, but this occurred in the winter months. March to June had an excess of 25.2 hours, July to September an

excess of 4.8 hours, the growing season thus having the closest approximation to the normal found in any year. (Table 10 and fig. 10.) The year consequently conforms very closely in all respects to the 50-year averages which make up the normal year.

It follows that in so far as climatic factors have determined its character and quality, the crop of 1920 should be a normal crop, having been produced under the influence of climatic factors in just the amount and with the distribution characteristic of the normal year. It does not follow that it was actually such in the sense in which the horticulturist employs the term, since he takes into account the effect of numerous nonclimatic factors in forming his estimate. These factors will be considered in a subsequent section, but it may be pointed out here that any departure of the 1920 crop from the orchardist's conception of a normal crop is to be attributed to non-climatic factors.

The remaining years each show very considerable abnormalities in amount or distribution of one or more factors. Because of certain similarities, 1921 and 1923 may be considered together. There were deficiencies of rainfall and excesses of temperature and sunlight in both, 1921 being much the hottest year of the period, while 1923 had the largest amount of sunshine in the growing season. The year 1921 was deficient in rainfall in the winter months and in the late summer, with almost normal precipitation from March 1 to June 30. Growth began very early, and the conditions of normal rainfall, considerable excess of sunlight (80.9 hours), and large excess of temperature (585°) combined to accelerate the development of a crop which had been materially reduced in size by frosts. Sunshine was nearly normal in late summer, but July was hot, August had only one-fourth the normal rain, and September was exceptionally hot. In consequence, the crop completed its development and reached maturity under conditions of severe heat and drought.

The year 1923 had a larger deficiency of rainfall than 1921, somewhat differently distributed in that the shortage of the winter was followed by a shortage in May and June, which had little more than half the normal amount. Winter temperatures were in excess of normal until February, but resumption of growth was not hastened and there was no injury from frost. The whole period from March to June, inclusive, was remarkable for its large excess of sunshine in every month, totaling 218 hours in the four months. Temperatures were normal until June, which was quite warm. The period beginning July 1 had practically normal heat units, the largest deficiency in sunshine (66.6 hours) of any year of the series occurring in this period, and there was a shortage in August and September rainfall.

Summing up the likenesses and differences of the two years, 1921 had favorable conditions for the development of the crop until midsummer, with high temperatures and shortage of rainfall as unfavorable conditions for its maturity. The year 1923 had favorable conditions until midsummer, except that a shortage of rainfall in May and June may have had injurious effects. The conditions under which the crop matured were decidedly more favorable than those of 1921, being more nearly normal in temperature and rainfall although somewhat deficient in sunlight.

The year 1925 may be considered in connection with 1921 and 1923 for the reason that it presents in greater intensity the condition of deficient precipitation. With an approach to the high temperatures of the late winter and spring months which were striking features of the season of 1921, it combines an excess of sunshine second only to that occurring in 1923. After July 1, 1925 had a deficiency of sunlight almost equaling that of 1923, combined with a temperature record like that of 1921 in that it remained close to normal until September, then rose markedly above the average. The season was thus a composite of the others, but with the factor of shortage of precipitation greatly intensified. A larger shortage for the winter months than in any other year was combined with a reduction of the amount received in March to June, inclusive, to less than half the normal. The crop consequently developed under conditions of excess light and heat and serious water shortage, and was matured under conditions of deficiency of sunshine, increasing deficiency of water, and exceptionally high temperature, especially in the last month of the season. Of the three years in which shortage of water supply may have been a limiting factor, 1925 is that in which it obviously must have played the most significant rôle.

The years 1922 and 1924 are similar in that there were considerable excesses of precipitation in both, but in its distribution and in that of the other factors there is very little likeness. Both years had winter temperatures considerably above normal, but the excess in 1924 was mainly due to a very warm December, the later months being near normal. The whole growing season of 1924 was one of consistently subnormal temperatures, ending with a deficiency of 355° F. The period from March 1 to July 1, 1922, had an average daily mean temperature 4.1° higher than 1924. Hours of sunlight closely approximated the normal in this period in both years, but 1922 had slightly subnormal precipitation in contrast to the very large excess of 1924. After July 1, 1922 received almost exactly the normal amount of heat, about one day less than normal hours of sun, and a large excess of precipitation. The year 1924 had a deficiency of 163° of heat and a small excess of sunshine. Torrential rains totaling 5.44 inches on September 29 and 30 give this period an apparent excess of 3.5 inches of rain, but when it is considered that no part of the precipitation of September 29 and 30 was available for the crop it is seen that the water actually available during the period was somewhat subnormal. Consequently 1922 was a year of rather high temperatures with excessive rainfall during the period of late development and maturity of apples, and 1924 was one of persistently subnormal temperatures with excessive precipitation in the first half of the growing period.

Summarizing, 1920 closely approached normality in all its climatic factors. The year 1921 was subnormal in having large excess of temperature throughout the growing season, with deficient rainfall in the latter half; 1922 had high temperatures in the first half of the growing season and excessive rainfall in the second half; 1923 had a large excess of sunshine and a material deficiency of water prior to July 1, with subnormal sunshine and rainfall after that date; 1924 had a large deficiency in total heat received during the season, with excessive precipitation in the first half; and 1925 was extremely hot and dry throughout, with an excess of sunshine prior to July 1 and a deficiency thereafter.

## RELATION OF SEASONAL CONDITIONS TO COMPOSITION OF THE CROP

In the preceding sections the climatic conditions during the period of the work have been discussed in considerable detail, and the analytical data have been considered with respect to the fluctuations in amount of various constituents occurring during the period. The analytical results show that the large group of varieties employed in the work display distinct mass tendencies, that is, they behave as one in very considerable degree in respect to the fluctuations in sugar, acid, and astringent substance occurring from year to year. It now remains to consider these mass tendencies, as they manifest themselves from year to year, in connection with the climatic data, and to ascertain in how far and in what way the character of the crop for each year, as measured by its composition, has been affected by the climatic conditions prevailing during the development of that crop. This can best be approached through a summation of the results of the analysis for the various years, followed by the consideration of the collective result for each year in relation to the climatic phenomena of that year.

Completeness of comparison of the analytical results is impossible, for the reason that the data upon sugar and acid content cover six years, 1920-1925, while data for astringent constituents are available for only the last four of these years. Consequently, any year may rank in any position from first to sixth with respect to sugar or acid content, but in only one of four positions in regard to astringent materials, since 1920 and 1921 can not be compared in this respect. The results are assembled in Table 13 so as to show the ranking of the various years with respect to the constituents mentioned.

TABLE 13.—Comparative rank of the crops of the six years 1920-1925 in respect to sugar, acid, and astringent constituents

[Rank of 1 indicates largest number of varieties having maximum content of the constituent indicated, 2 next largest, and so on. Comparisons are made for six years on total sugar and acid content and for four years on astringent substances]

Crop of—	Ranking of crop designated in—		
	Total sugar	Titrateable acidity	Relative astringency
1920.....	5	4	.....
1921.....	2	1	.....
1922.....	4	3	2
1923.....	1	2	4
1924.....	6	5	1
1925.....	3	6	3

It is immediately apparent from an inspection of Table 13 that there is a considerable degree of correlation between sugar, acid, and relative astringency.

The year 1923 has maximum sugar content and next to maximum acidity, associated with minimum relative astringency. The year 1924 has minimum sugar and next to minimum acidity, together with maximum relative astringency. The years 1922 and 1925, which are intermediate in sugar, are also intermediate in astringent material, though 1925 has minimum acidity. The year 1920 has low sugar and rather low acidity; 1921 has high sugar and maximum acidity, but data on astringent materials are lacking.

It is evident that sugar content and acidity are rather definitely coupled as to amount, and that the relation with relative astringency is one of antagonism, sugar and acid content tending to minimum values as astringency approaches maximum, or the reverse.

The consideration of the relation of climatic conditions to the results may be begun with 1923, a year of extremes. Maximum sugar content in a very large number of varieties was associated with high acid content and with reduction of relative astringency to the lowest level encountered during the entire period. The amounts of cane sugar present were maximum for the whole period of the analyses in a large majority of the varieties analyzed.

The climatic conditions of 1923, as summarized in Figure 12 (see also figs. 9, 10, and 11), were remarkable for the very large excess of sunshine (218.1 hours) combined with moderately high temperatures (180.5° F. excess) in the period from March 1 to June 30. This was succeeded in the period from July 1 to September 30 by practically normal temperature (17.2° excess) and materially subnormal sunshine (66.2 hours deficiency). The two parts of the season thus present the extremes in the matter of sunshine found in the series of years, the maximum in the first portion of the season and the minimum in the latter portion. It is clear that the additional opportunity for photosynthetic activity afforded the trees by the addition of 218 hours to the working time of the first four months is reflected in the large number of varieties having their maximum sugar content in that year.

The conditions prevailing during the period of maturity, namely, normal temperature and reduced sunshine, appear to have been conducive to the reduction of astringent nontannins to a low level, possibly by hydrolysis of glucosides and setting free sugar. In consequence, while true tannins were at the maximum level, nontannins were rather low, and relative total astringency was at the minimum.

That enzymic activity was relatively vigorous during the period of maturation is indicated by the fact that the samples of juice were as a group remarkably free of starch, as shown by the negative results of examination of the fresh juices as well as by the results of analyses of unfiltered Pasteurized samples. As compared with some other years in which starch was present in a considerable number of varieties, this indicates that diastatic activity was initiated earlier, or that it proceeded at a more rapid rate than in those years. Yet the amount of sucrose in the juices of this year were greatly in excess, both relatively and absolutely, of the amounts occurring in any other year for most of the varieties.

The acid content, while next to maximum in average amount, is materially below that of the maximum year both in absolute amounts and in comparative numbers of varieties having maximum amounts in that year. Acidity is apparently not so directly influenced by climatic conditions as are sugars and astringent substances, probably because its amount is due to the intensity of the respiratory process and to the oxygen supply, not to the amount of photosynthetic activity possible nor to the rate of enzymic hydrolysis.

The opposite extreme in chemical composition is observed in 1924, since that year has maximum relative astringency associated with minimum sugar and next to minimum acid contents. Examining the climatological summary (fig. 12), it is seen that the season up

to June 30 had practically normal sunshine (17.5 hours excess) but was notably deficient in heat received ( $192.2^{\circ}$  deficiency), while the remainder of the season had a like condition in sunshine (19.9 hours excess) with a further deficiency of  $163.4^{\circ}$  in temperature. The deficiency in temperature is very uniformly distributed over the whole growing season as an average deficiency of  $1.7^{\circ}$  per day. It acts as a brake or limiting factor upon photosynthetic activity, since carbohydrate content is at a minimum despite the excess of hours of sunshine received. The amount of sucrose in the juice is also low, the year ranking fifth in this respect. That the subnormal temperatures also reduced the rate of enzymic activity is indicated by the fact that many of the juices contained traces of starch, as determined by examination and by comparison of total sugars before and after Pasteurization. Such comparison shows that while starch was rather generally present, its amount was not sufficient to affect the ranking of the crop had it been determined and calculated as sugar.

Comparing the two extreme years, 1923 and 1924, it is found that their outstanding climatic differences consist in the reception in the first four months of the growing season of 1923 of 200.6 hours more sunshine and of  $372.7^{\circ}$  more heat than were received in the corresponding period in 1924. In the last three months 1924 had 86.5 hours more sunshine but  $180.6^{\circ}$  less heat than 1923. There was also a difference of 14.9 inches in rainfall, 1923 having a deficiency for the season of 4.78 inches, while 1923 had an excess of 10.12 inches; but there is scarcely a possibility that precipitation figured directly in the results. The large differences in the two crops must be attributed to the large differences in conditions for photosynthetic activity in the two seasons.

The year 1921 stands next after 1923 in point of sugar content, while it ranks first in total titratable acidity. The departures from the mean in climatic conditions consist in excesses of 80.9 and 15.9 hours of sunshine and of  $585.5^{\circ}$  and  $213.9^{\circ}$  of heat received in the first four and the last three months of the season, respectively. The excess of sunshine is materially less than that of 1923, but is accompanied by very much higher temperatures (nearly  $5^{\circ}$  per day excess). The two factors together are decidedly favorable to photosynthetic activity, raising the carbohydrate content of the fruit to a level second only to that attained in the less warm but phenomenally sunny season of 1923. In amount of sucrose present in the juice the year ranks second to 1923, but the amounts present were relatively and absolutely considerably below those found in that year.

The high carbohydrate content of the crop of 1921 is attributable to the seasonal conditions, the reduction in the size of the crop playing only a minor and insignificant rôle. This conclusion is stated with greater confidence by reason of the fact that the writer has reached it despite a preconceived opinion to the contrary. The crop upon many varieties was wholly destroyed or greatly reduced by frost. The possibility that the results might be vitiated by inclusion in the analyses of the fruit from trees markedly subnormal in crop was clearly in mind, and the varieties analyzed were restricted to those having 65 to 100 per cent of a full crop, as determined by persons familiar with the varieties concerned. A distinct majority of the varieties employed had crops equaling 85 per cent of the normal,

and only a relatively small proportion of the total number had crops falling between 65 and 75 per cent of full crop. Careful notes of yield of the individual varieties were made at the time of picking, and these notes have been exhaustively compared with the analytical data in an attempt to ascertain whether the varieties having a reduced load of fruit, as a group, show higher sugar content than the group having crops of normal or approximately normal size. No conclusive evidence of such differences can be found in any case in which the fruit on the tree equaled 75 per cent of normal, and such effects are not clearly or constantly present in those having the crop reduced below this level. That this is true is convincingly shown by the fact that exclusion from consideration of all varieties having less than 85 per cent of a full crop does not alter the rank of 1921 as to sugar content and makes only very insignificant changes in the percentages of the total number falling into first, second, and third places in respect to their sugar content.

This situation was exactly opposite to expectation and apparently in contradiction to prevalent conceptions as to the effects of thinning upon quality of crop. The explanation seems to lie in the differences between thinning due to frost and that performed by human agency. In the present case, as shown by the orchard notes, the frost injury was confined in most instances to the tops and northwest sides of the trees. On these portions there was usually only an occasional fruit, while the lower branches and the leeward side were fully loaded, only the central flower of most clusters having been killed. That a barren branch makes little or no contribution to the development of the fruit on other and distant portions of the tree is fairly well known. Consequently the effects of frost in this instance have nothing in common with the equalized distribution of fruit over the tree which is the purpose of thinning to accomplish. From these facts, and from the results of repeated and detailed examination of the data, the writer is convinced, contrary to his preconceived opinion, that the character of the 1921 crop was due to seasonal conditions without perceptible influence from frost thinning.

It is worthy of note that high acidity accompanies high sugar content in the years of high sunshine and high temperatures, 1923 and 1921, while minimum acidity accompanies minimum sugar in 1924, the year of markedly subnormal temperatures. The matter of acidity in relation to temperature will be referred to again in discussing the 1925 results.

The year 1920 has been commented upon as presenting an exceptionally close approximation to the normal or 50-year average in the distribution and amount of its climatic factors. It is fifth of the six years in point of sugar content and fourth in acidity. That it ranks so low may at first thought be surprising in view of its normal character. But it does not necessarily follow that the normal climatic conditions in the latitude of Washington are conducive to optimum development of apple fruits or to storage of a high content of sugar therein. It seems clear that the year is inferior in this respect to four of the five less normal years with which it is here compared. This must mean that in so far as photosynthetic activity as measured by the storage of sugar in apple fruits is concerned, a considerable increase above normal in hours of sunshine, as in 1923 and 1924, or in total temperature units received, as in 1922, or in both together, as in

1921, can be advantageously utilized by trees. It must mean also that the rainfall is considerably in excess of the minimum required by the trees, since considerable reduction in its amount, but not in its distribution, occurs in years showing higher sugar content than the normal. In other words, each of these factors has a value in the normal year considerably removed from the value at which it becomes a limiting factor, precipitation being considerably above, sunlight and temperature considerably below, the limiting values. Consequently, the fluctuations which have occurred in the amounts of these factors in four out of six years have been such as to favor greater storage of sugar; in one year only have they been such as to result in decreased sugar storage in the fruit.

It is especially unfortunate that data upon astringent constituents were not obtained, as it would be of decided interest to know the quantity of these constituents in a year conspicuous for the absence of outstanding departures from normal averages on the part of any climatic factor, as was 1920. That the crop as a whole contained the minimum amount of sucrose found in any year would indicate that the attainment of a high ratio of sucrose to total sugar in the fruit requires conditions not normally occurring at Washington.

The year 1922 is marked by its absence of extremes in the composition of the crop. In sugar content it stands fourth; in acid content third; in relative astringency it is second. The climatic conditions are in instructive contrast to those of 1920; 1922 had subnormal sunshine, 48.7 hours less than 1920, between March 1 and June 30, but this subnormal amount was associated with above-normal temperature as against subnormal in 1920. The difference between the two years is  $378.5^{\circ}$  for the four months, or more than  $3^{\circ}$  daily. The temperatures and hours of sunshine for the period subsequent to July 1 were almost identical for the two years, being very close to the normal. It would appear that the higher sugar content in 1922 must be attributed to the effect of the large additional number of heat units received despite the smaller number of hours of sunshine. Nontannins were maximum in amount (Table 6), pointing to a moderate rate of destruction under the normal temperatures prevailing during the last part of the growing season, and relative astringency ranks next to maximum by reason of the occurrence together of medium sugar and acid content and high astringent nontannins.

The year 1925 gave a crop showing a combination of chemical characters not elsewhere found during the period covered by the work. It is third in total sugar content, sixth in acid, and third in relative astringency. The crop was consequently lower in acid and astringency and higher in sugar than that of 1922, while higher in sugar, a little lower in acid, and much lower in astringency than that of 1924. The results, therefore, are in contradiction to those of these years and to the general situation found in all the other years, which is that of high sugar combined with low astringency or the reverse.

The seasonal conditions in the period from March 1 to June 30 present a combination of an excess of sunshine (151.8 hours) exceeded only in 1923 with a very considerable excess of heat ( $280.7^{\circ}$ ). The temperature excess approaches that of 1922, while the sunshine was 175.3 hours greater than in that year. If these factors alone determine the amount of photosynthetic activity, the sugar content



of the 1925 crop should be much greater than that of 1922 and should closely approach that of 1923. Instead it ranks only slightly above 1922 and very decidedly below 1923. The year 1925 had in the growing season 134.3 hours more sunshine and 472.9° more heat than 1924 and it had 126.6 hours more sunshine and 352.5° more heat than 1920, which should rank 1925 far above both and should show a large number of varieties attaining maximum sugar content. While 1925 does stand considerably above 1924 in this respect, it has percentages of maximum and next to maximum results only very slightly greater than 1920.

In the conditions subsequent to July 1, 1925 had a deficiency of sunshine almost identical in amount with that of 1923, with an excess of temperature over that year of 77.4° due to very high September temperatures. Such a combination can not be considered as responsible for the low sugar content, since the conditions of this period in 1923 were even less favorable for photosynthetic work, yet 1923 is the year of maximum sugar content.

These considerations force one to the conclusion that with respect to sugar content, 1925 stands one or two places below the rank in which the amount of sunshine and heat received would place it, and that photosynthetic efficiency has been considerably reduced by the operation of a limiting factor. That deficient rainfall may have been such a factor is at once suggested by inspection of Figure 12 and is very strongly indicated by Figures 9 and 8. The deficiency was not only greater in amount than in any other year, but it was so distributed as to exert maximum effect upon the crop. The shortage on March 1 was larger than in any other year, and the precipitation between March 1 and June 30 was less than half the normal, so that the total received during the winter and up to July 1 was only 1.77 inches more than the normal up to March 1. The crop began its development with a shortage of 5.45 inches, which mounted steadily to 13.32 at the end of July, then more slowly to 15.20 at the end of the season. The cumulative shortage approached its maximum in June, which was the hottest month of the year, with a mean temperature equaled in the records for Washington only by that of 1878, and with a large excess of sunshine (74 per cent of the possible instead of the normal 62). There was general evidence of suffering from water shortage on the part of farm crops in the plots surrounding the orchard and in the annuals growing in the orchard itself during the month of June, and flagging and incipient wilting of the foliage of the apple trees was often apparent. There is little doubt that partial or complete closure of the stomata with corresponding reduction or suspension of photosynthesis was of frequent occurrence during the period and possibly also in the excessively hot days of late May and early July. In consequence, the storage of sugar in the crop was reduced in an unmistakable degree below that which would have been possible with an adequate water supply.

Acid content reached the minimum for the whole period in 1925, while relative astringency was next to minimum for the 4-year period for which data were obtainable. The combination of low acidity and low astringency has not been encountered elsewhere in the series. Barss (6) has shown that in the case of the Bartlett pear, growth of the crop with an extremely limited water supply results in extremely marked and unpleasant astringency, and the writer has

observed that peaches grown without irrigation in the vicinity of Tucson, Ariz., are so astringent as to be almost inedible even when soft ripe. If a similar effect is produced in the apple, it occurs only when the deficiency of water is greater than was the case here or when other conditions are materially unlike those here encountered.

The low acidities of 1924 and 1920 occur in cool seasons in which the maturing of the fruit, as measured by its softening to cider ripeness after picking, was a rather slow process. The 1924 crop is also one characterized by high astringency. The situation in 1925 differs from that of these and other years in that the temperature during September was abnormally high, exceeding the mean by  $4.7^{\circ}$  daily, while that of October was abnormally low, the daily deficiency equaling  $5.3^{\circ}$ . (Fig. 8.) The transition from the mean temperature of  $72.8^{\circ}$  F. for September to  $52^{\circ}$  for October is in decided contrast with the normal change from  $68.1^{\circ}$  to  $57.3^{\circ}$ . The high temperatures of September accelerated maturity changes, so that picking occurred somewhat early in the case of many varieties. In the case of early varieties, softening went on rapidly after picking, and fruit could be held for only very short periods before it reached pressing ripeness. The later maturing varieties were similarly affected by September conditions, beginning to soften either on the trees or in storage, but as the cooler October weather came on there was a marked slowing down in rate of softening. This was especially evident in fruit held in a basement room after picking, many varieties showing very little or no change in firmness, as measured by a puncture test, during 10 to 15 days. The effect of the high temperatures of September was undoubtedly to accelerate respiration and enzymic processes, such as starch conversion and the splitting of glucosides. With the large alteration of temperature which occurred near the beginning of October, these processes were greatly slowed concurrently with the slowing down of softening of the tissues.

The change occurring under these conditions was somewhat like that which may be produced by placing fruit in cool storage. That some such effect would follow was anticipated, and quantities of 10 varieties were placed in a basement room and sampled at intervals of about 10 days during late October and early November. The results, presented in Table 14, show quite clearly what happened. Titratable acidity is rather low at the first sampling, rises somewhat, then drops rather rapidly. Starch conversion goes on rapidly at first, then much more slowly. Nontannin astringency decreased irregularly, but rather more rapidly at the outset than later. It had already reached a rather low point before storage. The effect of the incipient softening of the tissues in some way increased the subsequent rate of disappearance of acid, probably by increasing the permeability of the tissues to oxygen. Magness and Burroughs (27) found that high respiratory rate reduced acid content much more rapidly than sugar. The general behavior of these lots of fruit is that of fruit allowed to become overripe before picking and placing in storage. The rapid changes initiated in the fruit while still on the trees were somewhat slowed down, but not to the degree which would have occurred in a year having less marked departures from the normal temperature curve.

TABLE 14.—Results of repeated analyses at short intervals of fruit held in common storage, October and November, 1925

Variety	Date picked	Date analyzed	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar	Acid	Total astringency	Tannin	Non-tannin	Solids
Akin.....	Oct. 15	Oct. 22	7.05	2.63	9.68	0.260	0.1055	0.0502	0.0553	11.79
		Oct. 31	8.48	2.80	11.28	.314	.0772	.0212	.0560	13.56
		Nov. 10	9.00	2.76	11.76	.268	.1062	.0383	.0679	13.56
		Nov. 20	8.64	2.57	11.21	.230	.1011	.0342	.0669	12.84
Baldwin.....	do	Oct. 22	4.66	2.94	7.60	.268	.0740	.0177	.0563	10.06
		Nov. 2	7.33	3.12	10.45	.390	.0900	.0373	.0527	12.74
		Nov. 10	7.03	2.80	9.83	.351	.0940	.0420	.0520	11.32
		Nov. 21	6.84	2.79	9.63	.314	.0876	.0364	.0512	10.96
Ben Hur.....	Oct. 10	Oct. 22	5.34	2.29	7.63	.188	.0537	.0147	.0390	10.13
		Nov. 2	7.35	2.99	10.34	.196	.0832	.0357	.0475	12.49
		Nov. 10	7.11	2.69	9.80	.173	.0710	.0294	.0416	11.96
Hort. No. 3050.....	Oct. 16	Oct. 21	8.37	1.52	9.89	.292	.1440	.0512	.0928	12.75
		Oct. 31	9.68	1.76	11.44	.242	.1260	.0495	.0765	14.70
		Nov. 9	9.40	1.46	10.86	.260	.1140	.0255	.0885	14.34
Keeper.....	Oct. 10	Oct. 22	5.98	2.82	8.80	.334	.0366	.0066	.0300	9.67
		Nov. 2	7.42	2.62	10.04	.380	.0495	.0095	.0400	12.07
		Nov. 15	6.94	2.39	9.33	.331	.0408	.0058	.0350	11.69
Kinnard.....	Oct. 3	Oct. 7	5.76	2.66	8.42	.404	.0835	.0360	.0475	10.43
		Oct. 22	6.36	1.96	8.32	.280	.1062	.0432	.0630	10.22
		Oct. 31	7.54	2.09	9.63	.314	.0896	.0302	.0597	12.19
		Nov. 10	6.86	1.62	8.48	.260	.1062	.0438	.0624	10.80
Santa.....	Oct. 16	Oct. 22	6.48	2.61	9.09	.710	.0950	.0380	.0570	11.69
		Oct. 31	6.66	2.92	9.58	.628	.0722	.0230	.0492	12.18
		Nov. 9	6.19	2.53	8.72	.645	.0892	.0272	.0620	10.61
Shone.....	Oct. 3	Oct. 21	8.44	2.49	10.93	.292	.0485	.0120	.0365	13.61
		Oct. 31	8.78	2.72	11.50	.374	.0638	.0188	.0450	13.76
		Nov. 9	8.44	2.50	10.94	.378	.0706	.0289	.0417	12.86
		Nov. 18	8.23	2.18	10.41	.331	.0674	.0266	.0408	12.67
Sweet Orange.....	Oct. 10	Oct. 22	7.54	3.45	10.99	.150	.0895	.0257	.0638	12.21
		Nov. 2	10.16	3.65	13.81	.166	.1110	.0370	.0740	16.63
		Nov. 19	9.83	3.61	13.44	.141	.1011	.0383	.0628	16.41
Winter Paradise.....	do	Oct. 21	8.70	1.76	10.46	.104	.1699	.0650	.1049	12.45
		Oct. 31	9.32	2.16	11.48	.092	.1620	.0670	.0950	13.84
		Nov. 9	8.62	1.89	10.51	.078	.1530	.0710	.0820	12.88

Until much more is known as to the nature of the astringent materials and the character of the changes that they undergo during the maturation of the fruit, it is impossible to offer anything more than a tentative explanation of the fact that high sugar and acid content are correlated with low relative astringency, or the reverse. The present study yields some data toward the formation of such an explanation. A considerable portion of the material included in the astringent nontannins consists of flavones and related compounds, chiefly glucosidal in nature. These are greatest in amount in the immature fruit and gradually decrease as maturity proceeds. The processes responsible for the decrease are in part hydrolysis and in part physical combinations with pectins as these are brought into solution from the cell walls. Temperature controls the rate of both these processes, determining the rate of the hydrolysis of glucosides and also the rate of solution of pectins. The presence of oxygen is not necessary for either process. High temperatures during the ripening of the fruit appear to be definitely associated with low astringency, as in 1923

and 1925, and low temperatures in this period with high astringency, as in 1922 and 1924. It has already been noted that the amount of starch present in the fruit at the stage of cider ripeness is greater in years having low temperatures during the ripening period. This would indicate that diastase is less abundant or less active in the cooler seasons. If it be true that temperature controls the rate of these enzymic processes, the presence of starch and of high astringent content in the years of lower temperatures in the ripening period, and the absence of starch and reduction of astringent substances to a low level in the years of warm harvest seasons, are thereby explained.

#### GENERAL DISCUSSION

It has been shown in the discussion of the analytical data that, in the apple, sugar content is rather definitely and consistently correlated with acidity and astringency, positively with acidity, negatively with astringency, so that high sugar and high acid are accompanied by low astringency, and low sugar and low acid by high astringency. These correlations break down only under the exceptional condition that a climatic factor is so far altered in amount that it becomes limiting and thereby alters the course of some physiological process while permitting others to go on unaltered or possibly accelerated. Such exceptional conditions were encountered in 1925, but with the exception of this year the correlations just stated hold quite well for the large group of varieties here considered, which purposely includes fruits showing as wide range in character as it was possible to obtain.

It must be emphasized that the correlation here stated is not to be understood as a hard-and-fast rule which will apply without exception to individual varieties selected at random from the analytical tables. It is not of this character, and any attempt so to apply it will encounter numerous individual cases in which it does not hold. The conception which is intended to be conveyed by the statement that high sugar, high acidity, and low astringency are associated, and that low sugar, low acidity, and high astringency are also found together, is the conception that in a broad way these chemical situations are apparent when a large group of varieties are considered en masse. The climatic factors operating upon the developing crop are exerting an effect upon the quantities of these three constituents which will be evident when the crop is analyzed at any given moment of its maturity. The conditions which make for attainment of high sugar content are those which make for retention of acidity at a high level and also those which make for reduction of astringent materials to a low level. Conversely, the conditions which tend to hold the sugar content to a low level exert a like effect upon acid content, while tending to leave astringency at a high level.

These effects will be apparent as mass tendencies when the data for a large number of varieties are collectively examined, and the direction and extent of the mass movement will be found to be controlled by the character of the environmental complex. But the individual varieties will not all exhibit the same tendencies or exhibit them to an equal degree, for two reasons. In experiments in which all the conditions are as carefully controlled as is possible, a group of apparently identical plants of the same species or variety show individual variations to an extent which makes it necessary to work with large

numbers and obtain average results. In the present work a great diversity of uncontrolled conditions exists. Differences of soil texture and moisture content, exposure to sun, age, physical vigor, freedom from disease, and fruiting habits of trees are some of the factors which are not controlled. That the response to any given condition on the part of such an assemblage will not be unanimous in character or amount is certain, because of the great variety of these unmeasured differences between individuals. It is precisely because such a heterogeneous assemblage of plant forms behaves in such large degree as one in its response to differences in the seasonal conditions that one may feel confident that he is dealing with a general and fundamental fact with respect to the response of plants to the environment. The response of the occasional individual may be modified or suppressed by conditions peculiar to itself, but the response of the group as a whole may safely be taken as the expression of a truth.

As has been pointed out by the writer, in discussing the results of his work upon grapes (11), the composition of a fruit at maturity is the resultant of a series of interrelated physiological processes. Climatic conditions may influence two of these processes in the same direction, but not to the same degree, since each process is conditioned as to its rate by other factors. Nor will the progress of any one process in two different plants or fruits necessarily be identical, since it is in some degree affected by internal conditions peculiar to the individual. Consequently it is not to be expected that high sugar content is in all cases to be found associated with low astringency, but rather that this will be so frequently the result that we are forced to the conviction that the conditions which produce the one result are also in the main productive of the other.

It is to a very considerable extent true that individual varieties show the same correlations between acid, tannin, and sugar that have been shown to hold for groups of varieties. The analyses of Shaw (30) show a fairly consistent correlation between acid and sugar content, which are high or low together. When it is considered that Shaw's samples were collected at widely separated points and by various individuals, subjected to widely varying conditions during transmission to Amherst, and analyzed at various stages of maturity, it is rather surprising that the relation of acid to sugar was as consistent as the figures show it to be. Unfortunately, no determinations of astringent constituents were made. Jones and Colver (22) made a very extensive series of analyses of apples and other fruits grown in Idaho. The primary purpose of their work was to ascertain whether there are constant differences of composition between fruits of the same variety when grown on irrigated and nonirrigated soils. The samples analyzed were collected from all sections of the State, the accompanying data relating only to the irrigation or absence of irrigation of the trees. Under the conditions there was necessarily considerable variation in the handling and treatment of the material and consequently in the condition of the various samples, although all were characterized as fresh ripe at the time of analysis. An examination of the results of these investigators is nevertheless of considerable interest. They analyzed 284 samples of 17 standard varieties, the number of samples of a variety ranging from 3 to 79. The samples were collected in 1909, 1910, and 1911. There is throughout their work a quite consistent association of high sugar

content with high acidity and of medium or low sugar with medium to low acidity, in both irrigated and nonirrigated fruits. The absolute maxima occur together in a number of cases, as do absolute minima, and the two constituents take rank near together in all but a small number of cases.

Jones and Colver did not make determinations of tannins and astringent materials, and it has not been possible to find in the literature any series of analyses which would throw light upon the relation of these constituents to acidity and sugar content. Bigelow, Gore, and Howard (8), Alwood, Davidson, and Moncure (4), and others who have made determinations upon tannin in connection with ripening processes or in other studies, do not have data with a bearing upon the point here under consideration.

In his work on the effects of climatic conditions upon the chemical composition of grapes, the writer found a rather clear-cut and consistent correlation of a different type. The nearness of the ocean has a stabilizing effect upon summer temperatures at Vineland, N. J., where the work with grapes was carried on, with the result that the amount of sunshine received during the growing season was the only climatic factor showing large annual variations during the period of the work. The sugar content of the crop varied with the amount of sunshine received, being highest in the year of maximum sunshine and decreasing with decrease in the amount of that factor. Acid and astringent content were correlated with sugar content, but in such fashion that high sugar was accompanied by low acid and astringency, and low sugar by high acid and astringency. In the grape, acid and astringent content take rank together, always in opposition to sugar content; in the apple, sugar and acid take rank together in opposition to astringency content. In the grape, the total astringency, that is, the absolute amount of astringent materials, varies in the manner stated; in the apple it is not the absolute but the relative total astringency, that is, the amount of astringent material considered in its relation to sugar and acid content, which varies in this way.

The unlikeness of the results with respect to acidity in the apple and the grape is to be anticipated in view of the work of Alwood (2, 3) on the changes in acidity in grapes during ripening. In the grape, the acid of the immature berry is rather sharply localized in two areas, one about the seeds, the other a zone of pulp immediately beneath the skin, as Hedrick (20) has shown. During the ripening process, conversion of the acid of the peripheral layer into tartrates occurs, the disintegrating pulp becoming filled with tartrate crystals (2). In a number of varieties studied by Alwood, the titratable acidity of the juice was reduced during the ripening period to less than half that present at its beginning (3). Disintegration of the peripheral zone of pulp facilitates tartrate formation by permitting contact of the reacting substances, and the rate at which this disintegration occurs is determined by the climatic conditions of the ripening period. High temperatures and continuous sunshine consequently favor reduction of titratable astringency through accelerating formation of acid salts. In the apple there is no comparable transformation of acids during ripening.

In the grape the materials contributing to total astringency are, except in occasional nonpigmented varieties, mainly nontannins, that is, coloring matters, glucosides, and other substances oxidiz-

able by potassium permanganate but not precipitable with gelatin. In consequence, whether total astringency in any given grape shall be high or low is determined by the amount of astringent nontannins present, the proportion borne by the true tannins to the total being too small to affect materially its amount. In the apple the amount of true tannin in relation to the total astringent material present is usually much larger than in grapes, and in many varieties it makes up the larger part of the total.

Tannin fluctuates considerably in amount in the apple from year to year, apparently as a variable not directly affected by the amount of any other constituent, and these fluctuations affect the sum total of astringents much more than is the case in the grape. The result is that absolute amounts of astringents are as often determined by the tannin as by the nontannin fraction. Nevertheless, relative astringency, that is, the ratio borne by the amount of astringent substances to sugar content, exhibits the same correlation with sugar and acid found for total astringents in the grape.

It has been stated above that tannin content in the apple appears to be a variable. The quantity present in the fruit of any variety of apple from year to year appears to fluctuate without discernible relation to the intensity or distribution of any factor of the environment. During the preparation of this paper the writer has examined the data of his previously published paper upon grapes, and is convinced that tannin content in the grape is also an independent variable.

It would appear, therefore, to be a tenable conclusion that the seasonal amount and distribution of the climatic factors sunshine, temperature, and precipitation affect the composition of the crop borne by apple trees grown under controlled conditions in definite and consistent fashion. That the response to variations in amount of these factors is a fundamental physiological one is evidenced by the fact that a group of 216 varieties, in which apples of the greatest obtainable diversity in type and character were purposely included, shows a definite mass behavior or tendency to behave as a unit through six successive years. This mass behavior exhibits a definite relation to the variations in climatic conditions during the growing season. That the response is a fundamental response of the plant mechanism to the conditions of the environment is further shown by the fact that under like seasonal conditions the apple and the grape show like modifications in chemical composition. The two fruits exhibit the same correlation between the changes in amount of their constituents, with minor differences attributable to their structural and physiological dissimilarity.

#### GENERAL SUMMARY

The apple variety collection of the Bureau of Plant Industry at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., has been employed in a study of the variations in chemical composition of the expressed juices occurring from year to year and the relations borne to such variations by the annual variation in climatic conditions. The trees received uniform cultural treatment during the six years of the work (1920-1925). Of the varieties included in the experiment at its beginning, 216 fruited with a sufficient degree of regularity to permit inclusion of the data in the

results. These 216 varieties include apples of the widest diversity in character of fruit and adaptation to locality possible to procure in the collection.

The six years during which the work was in progress presented an exceptionally wide range both in the amounts and distribution of the individual climatic factors and in the combinations of these factors occurring during the growing season at Washington. The year 1920 was remarkable for the close approximation to the 50-year average in the amount and distribution of the three climatic factors, sunshine, temperature, and precipitation; 1921 was an exceptionally warm year, receiving 799.4° excess temperature in the period from March 1 to September 30, with a considerable excess of sunshine; 1922 had a considerable excess of temperature and of precipitation, with less than normal sunshine; 1923 had a very large excess of sunshine, rather high temperature, and subnormal rainfall; 1924 was markedly subnormal in temperature, had a large excess of precipitation, and a little more than normal sunshine; 1925 was excessively dry with consistently above-normal temperatures and considerably more than normal sunshine.

The chemical composition of the crop of a given year, when the results for all varieties are considered collectively, shows a definite relationship to the amount and distribution of the climatic factors during the growing season of that year. These climatic factors exert an effect upon chemical composition which manifests itself as modification in like direction and degree of the composition of the fruit of a large number of varieties of widely diverse character and degree of adaptation to locality.

Of the three climatic factors, sunshine, temperature, and precipitation, variations in amounts of sunshine and temperature, within the limits encountered in this work, are most effective in determining the chemical composition of the crop at Washington, D. C. The fact that the amounts of sunshine and heat received departed from the normal in the same direction in three years and in opposite directions in the remaining three permits an approximate relative evaluation of their separate effects. Variations from normal in amount of sunshine received during the growing season are productive of largest effect; variations in amount of heat received are less effective, within the limits encountered in this work. Precipitation may depart very considerably in either direction from the normal at Washington without discoverable effect upon the crop. The amount and distribution of rainfall is a significant factor in determining the composition of the crop in one year only of the six years covered by the work.

If the composition of the crop in 1920, a year very closely approximating the normal in climatic conditions, is used as a basis for comparison, the relation of climatic conditions to chemical composition of apples at Washington is such that increases in amount of sunshine and temperature over the 50-year average or normal amounts result in general increases in sugar content of the fruit over the amount present in the climatically normal year. Decreases in amounts of sunshine and temperature below the 50-year average result in general depression of sugar content of the fruit below the amount present in the climatically normal year. The extent of the departure of sunshine and temperature from the normal amounts is a direct measure



of the departure of the composition of the crop from that of the climatically normal year in every year except 1925. In that year, a large deficiency in precipitation operated as a limiting factor upon photosynthesis and altered the degree but not the direction of the response of the crop.

There is a definite and consistent correlation of sugar content, both of total sugar and of sucrose, with acid content and content of astringent substances. The correlation between sugar content and acidity is positive, that between sugar and astringent substances is negative, high sugar content being accompanied by high acidity and low astringency, low sugar content by low acidity and high astringency, and medium sugar content by absence of extremes in amounts of the other two constituents. This general relationship of sugar, acid, and astringent content is consistently presented by masses of varieties. In one year the correlation breaks down as a result of the interference of a limiting factor, shortage of water, with the normal course of physiological processes.

The correlation between sugar, acidity, and astringent content just stated is not an absolute and rigid rule, applying without exception to individual varieties under the conditions of the experiment. There are a large number of known and unknown conditions, such as local differences in soil, water supply, exposure, individual or varietal fruiting habit, susceptibility to disease, and others which can not be measured or brought under control in large-scale field experiments of this character, and which produce a considerable number of individual exceptions. That the correlation is a mass phenomenon consistently exhibited over a series of years despite the effect of these uncontrolled factors is conclusive evidence of its fundamental character.

The climatic conditions conducive to the development of a high content of sugar and acid in the fruit of the apple are also conducive to the reduction to a low level of the content of astringent materials therein. Conversely, the climatic conditions which limit the development of sugar and acid content to a medium or low level are such as increase the formation, or depress the rate of destruction, of astringent substances, thus leaving their amount at a higher level. As a result, absence of extremes, as medium sugar and medium astringency, or opposite extremes, as high sugar and low astringency or the reverse condition, are found together. Like extremes, as low sugar and low astringency, are not found together, for the reason that any one set of climatic conditions can not produce both results. In consequence, the acid-astringency-sugar ratio of a given variety will vary quite widely from year to year in a locality having as wide variation in seasonal conditions as Washington, and will have relatively narrow variations in a region having a narrow range of variation in seasonal conditions.

The acid-astringency-sugar ratio, which states the relative amounts of titratable acidity, total sugars, and total astringent substances contained in a fruit juice, presents the results of chemical analysis in a form designed to convey a conception of the beverage quality of the juice. The ratio expresses the relative amounts of the three factors upon which the sense of taste bases its estimate of the product. Since beverage quality depends upon the relation rather than the absolute amounts of these substances, a knowledge of the acid-

astringency-sugar ratio is more important than a chemical analysis in determining the beverage quality of a juice.

It has now been established that climatic conditions during the growing season exert a determining influence upon chemical composition of the annual crop of fruit in the case of two perennial plants, the apple and the grape, and that the character of the effect upon the various constituents of the fruit is identical except for secondary differences due to morphological dissimilarities of the two fruits. Since large groups of dissimilar varieties of the two species exhibit uniformity of response to the annual variations in the factors of the climatic complex, it would appear that the annual crop of fruit upon a perennial plant is an integrated expression of the climatic factors for the season in which it is produced, in the same degree to which the growth of an annual plant integrates these factors.

#### LITERATURE CITED

- (1) ABBE, C.  
1905. A FIRST REPORT ON THE RELATIONS BETWEEN CLIMATES AND CROPS. U. S. Dept. Agr., Weather Bur. Bul. 36, 386 p.
- (2) ALWOOD, W. B.  
1911. ENOLOGICAL STUDIES. THE SUGAR AND ACID CONTENT OF DIFFERENT VARIETIES OF GRAPES, SAMPLED AT FREQUENT INTERVALS DURING RIPENING AND AT FULL MATURITY. U. S. Dept. Agr., Bur. Chem. Bul. 140: 16-24.
- (3) ———  
1914. CRYSTALLIZATION OF CREAM OF TARTAR IN THE FRUIT OF GRAPES. Jour. Agr. Research 1: 513-514.
- (4) ——— DAVIDSON, W. J., and MONCURE, W. A. P.  
1904. CHEMICAL COMPOSITION OF APPLES AND CIDER. U. S. Dept. Agr., Bur. Chem. Bul. 88, 46 p.
- (5) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1920. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C.
- (6) BARSS, A. F.  
1915. THE PEAR AS AFFECTED BY MOISTURE SUPPLY. Oreg. Agr. Expt. Sta. Bien. Crop Pest and Hort. Rpt. (1913/14) 2: 38-49, illus.
- (7) BEACH, S. A., BOOTH, N. O., and TAYLOR, O. M.  
1905. THE APPLES OF NEW YORK. 2 v., illus. New York.
- (8) BIGELOW, W. D., GORE, H. C., and HOWARD, B. J.  
1905. STUDIES ON APPLES. I. STORAGE, RESPIRATION, AND GROWTH. U. S. Dept. Agr., Bur. Chem. Bul. 94: 1-67, illus.
- (9) BUNYARD, E. A.  
1920. A HANDBOOK OF HARDY FRUITS MORE COMMONLY GROWN IN GREAT BRITAIN. APPLES AND PEARS. 204 p., illus. London.
- (10) CALDWELL, J. S.  
1922. STUDIES IN THE CLARIFICATION OF UNFERMENTED FRUIT JUICES. U. S. Dept. Agr. Bul. 1025, 30 p.
- (11) ———  
1925. SOME EFFECTS OF SEASONAL CONDITIONS UPON THE CHEMICAL COMPOSITION OF AMERICAN GRAPE JUICES. Jour. Agr. Research 30: 1133-1176, illus.
- (12) ———  
1928. MEAN SUMMER OR "OPTIMUM" TEMPERATURES IN RELATION TO CHEMICAL COMPOSITION IN THE APPLE. Jour. Agr. Research, 36: 367-389.
- (13) CANDOLLE, A. DE  
1855. GÉOGRAPHIE BOTANIQUE RAISONNÉE. 2 v., illus. Paris.
- (14) CHANDLER, W. H.  
1925. FRUIT GROWING. 770 p., illus. Boston, New York [etc.].
- (15) DUGGAR, B. M.  
1911. PLANT PHYSIOLOGY, WITH SPECIAL REFERENCE TO PLANT PRODUCTION. 516 p., illus. New York.

- (16) GANTTNER, F.  
1889. DIE BESTIMMUNG DES GERBSTOFFES MIT CHAMÄLEON. *Ztschr. Angew. Chem.* 1889: 577-580.
- (17) GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D.  
1922. THE FUNDAMENTALS OF FRUIT PRODUCTION. 686 p., illus. New York.
- (18) GOURLEY, J. H.  
1917. SOME OBSERVATIONS ON THE GROWTH OF APPLE TREES. *N. H. Agr. Expt. Sta. Tech. Bul.* 12, 38 p., illus.
- (19) HABERLANDT, F.  
1874. DIE OBEREN UND UNTEREN TEMPERATURGRENZEN FÜR DIE KEIMUNG DER WICHTIGEREN LANDWIRTSCHAFTLICHEN SÄMEREIEN. *Landw. Vers. Sta.* 17: 104-116.
- (20) HEDRICK, U. P.  
1919. MANUAL OF AMERICAN GRAPE-GROWING. 458 p., illus. New York.
- (21) HOWARD, W. L.  
1915. AN EXPERIMENTAL STUDY OF THE REST PERIOD IN PLANTS. FIFTH REPORT. *Mo. Agr. Expt. Sta. Research Bul.* 21, 72 p., illus.
- (22) JONES, J. S., and COLVER, C. W.  
1912. THE COMPOSITION OF IRRIGATED AND NON-IRRIGATED FRUITS. *Idaho Agr. Expt. Sta. Bul.* 75, 53 p.
- (23) KELHOFER, W.  
1908. BEITRÄGE ZUR KENNTNIS DES BIRNGERBSTOFFS UND SEINER VERÄNDERUNGEN BEI DER OBSTWEINBEREITUNG. *Landw. Jahrb. Schweiz* 22: 343-410, illus.
- (24) LIVINGSTON, B. E.  
1916. PHYSIOLOGICAL TEMPERATURE INDICES FOR THE STUDY OF PLANT GROWTH IN RELATION TO CLIMATIC CONDITIONS. *Physiol. Researches* 1: 399-420.
- (25) ——— and LIVINGSTON, G. J.  
1913. TEMPERATURE COEFFICIENTS IN PLANT GEOGRAPHY AND CLIMATOLOGY. *Bot. Gaz.* 56: 349-375.
- (26) MCLEAN, F. T.  
1917. A PRELIMINARY STUDY OF CLIMATIC CONDITIONS IN MARYLAND, AS RELATED TO PLANT GROWTH. *Physiol. Researches* 2: 129-208.
- (27) MAGNESS, J. R., and BURROUGHS, A. M.  
[1923]. STUDIES IN APPLE STORAGE. *In* STORAGE INVESTIGATIONS, 1921-1922. *Marble Lab. Inc. Rpt.* 2: 17-98, illus. Canton, Penn.
- (28) MERRIAM, C. H.  
1898. LIFE ZONES AND CROP ZONES OF THE UNITED STATES. *U. S. Dept. Agr., Bur. Biol. Survey Bul.* 10, 79 p., illus.
- (29) NEUBAUER, C.  
1872. STUDIEN ÜBER DEN ROTHWEIN. *Ann. Oenologie* 2: 1-41.
- (30) SHAW, J. K.  
1911. CLIMATIC ADAPTATIONS OF APPLE VARIETIES. *Mass. Agr. Expt. Sta. Ann. Rpt.* 23: 177-245, illus.
- (31) UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU.  
1920-25. MONTHLY METEOROLOGICAL SUMMARY, WASHINGTON, D. C. Form 1030. Washington, D. C.
- (32) ———.  
1917-1925. CLIMATOLOGICAL DATA, VIRGINIA SECTION. *U. S. Dept. Agr., Weather Bur. Climatol. Data, Va. Sect.*, vols. 27-35. Richmond, Va.



# MEAN SUMMER OR "OPTIMUM" TEMPERATURES IN RELATION TO CHEMICAL COMPOSITION IN THE APPLE<sup>1</sup>

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## INTRODUCTION

The extent and character of the alterations in chemical composition which the fruits of a given variety of apple may undergo when the trees are grown over a wide range of latitude are very imperfectly known. The relative importance of character of soil, cultural treatment, and climatic conditions in the production of such differences as are observed when comparisons are made between fruits of the same variety grown in widely separated districts is exceedingly difficult to determine, since these factors vary independently in each of the districts under comparison.

The only experimental studies to be found in the literature of the effect on apples of growth under widely varied climatic conditions are those of Shaw (10, 11).<sup>2</sup> The results of his investigations led him to consider summer temperatures as the predominant factor in determining differences in chemical composition of the fruit and resulted in the formulation of the theory that every apple variety has an optimum mean summer temperature at which it attains its best development, departing in a definite manner from such development with departure of the mean temperature from its optimum.

## THE OPTIMUM-TEMPERATURE THEORY

Shaw carried on a rather extensive investigation, the results of which are reported in two papers. The first of these (10) dealt with the range in form, size, dessert quality, and other characters in the Ben Davis apple in a single orchard, that of the Massachusetts Agricultural Experiment Station, as compared with the variations in these characters found in more than 9,000 specimens obtained from 32 localities representing the entire range of the variety from Quebec to Texas, Arkansas, and California. A progressive alteration in form associated with lessened juiciness, greater astringency, and lower dessert quality was found in the northern-grown samples and was attributed to differences in climatic conditions, temperature being considered the controlling factor. The second paper (11) reports the result of extension of the study to include 19 varieties. Samples of these were collected from a number of localities, ranging from 2 or 3 in the case of the less widely grown varieties to 10 to 15 in those of wider distribution, but so chosen as to represent in so far as possible the range of the variety.

<sup>1</sup> Received for publication July 28, 1926; issued April, 1928. This paper is the fourth in a series of studies on fruit juices.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 388.

Ben Davis was represented by 15 samples from 8 States and 3 Canadian Provinces; York Imperial by 8 samples from 6 States; Winesap by 9 samples from 7 States; Baldwin by 11 samples from 6 States; Northern Spy by 10 samples from 7 States and Provinces; Grimes Golden by 11 samples from 7 States and British Columbia; and Jonathan by 10 samples from 8 States and Provinces. The samples were assumed to represent the extent of variation of the varieties to be found throughout the range in which they can be successfully grown. It was further assumed that any particular sample was truly representative of the variety as grown in the latitude from which it came, and that the climatic conditions of the locality and year were in every case typical for the latitude represented by the sample.

The samples were collected by growers in the various places of origin. The samples, ranging from half a dozen fruits to a barrel in quantity, were shipped to Amherst, placed in cold or cellar storage, and analyzed at various times from November to March. The analyses upon the samples of any one variety were made at irregular intervals throughout this period, not all at one time.

The determinations made included total solids, insoluble solids, sucrose, reducing sugars, and acidity. Notes on color and storage and dessert quality were made in some detail. The analytical data are made the basis for formulating several conclusions. These may be briefly summarized:

1. The climatic factor is more potent in producing variation in apples than differences in soils or in cultural treatment.

2. Every variety has an optimum mean summer temperature at which it attains its best development. When grown at either higher or lower temperatures it deteriorates in dessert quality. At lower temperatures the chemical and physical differences are those characteristic of immaturity and include greater acidity and astringency, increased insoluble solids, decreased size and color, and tendency to scalding in storage. Grown at higher temperatures, the fruit is characterized by uneven ripening, premature dropping and rotting on the tree, poor keeping quality, lack of flavor, "mealiness," decreased size, and less intense color. Shaw considered that a difference of 2 degrees or more from the mean optimum temperature in the case of any variety would result in the modification of the fruit in the corresponding direction.

3. The apples on the list of highly successful varieties prepared by the American Pomological Society, with a number of additions, to a total number of 165, are subdivided into 16 groups, to each of which an optimum mean summer temperature (obtained by averaging the monthly means for the seven months March to September) is assigned. These optimum temperatures range from 52° F. for Hiberna, Okabena, and Oldenburg to 67° for Terry and Yates. The largest groups are those with optimum temperatures between 54° and 60°, which have 12 to 20 varieties each.

Shaw's optimum temperature groupings have been widely quoted in textbooks upon fruit growing (3, 4, 5, 9). In some of the older books they are made the basis of a recommendation that the grower select varieties for planting with consideration of the relation of their optimum temperatures to the mean summer temperature prevailing in his locality. There is a growing tendency, expressed by Chandler (3), to regard the temperature ranges within which optimum development of a variety can occur as somewhat wider than those set by Shaw, but no experimental work has been done since the appearance of his papers.

That the amount of heat received during the growing season, apart from all other factors, can be the only significant factor in

determining the chemical composition of the fruit would appear somewhat questionable. That mean summer temperatures are a very unsatisfactory index of the actual temperature conditions during the season is apparent from a consideration of the methods usually employed in arriving at such means. At most weather stations the daily mean is obtained by halving the sum of the readings of a maximum and minimum thermometer. The sum of such daily means for the entire season, divided by the number of days, is the seasonal mean. Such a figure may be identical for two stations having very different actual conditions. It may be very different from that which would be obtained from averaging hourly temperatures over the same period, and may lead to a quite erroneous idea of the quantity of heat received during the period. In any case the statement of seasonal mean temperature gives no idea of the distribution of the heat received over the period of activity of the plant, and consequently can convey no conception of the part temperature has played in the various phases of vegetative development and production of fruit. It was considerations of this character which led Livingston and Shreve to say (8, p. 206):

Mean temperatures for long periods of time are not apt to be of value in studies of the relations between plant activities and the environment; seasonal or yearly means, which comprise such an important part of the usual meteorological reports, seem never to have given any real promise in this direction. Such means do not take account of the duration factor \* \* \*.

These authors abandoned the use of mean temperatures in favor of the method of physiological indices of temperature efficiency for plant growth as developed by Livingston (7) from the work of Lehenbauer (6).

In view of the fact that the standard employed by Shaw in classifying the varieties studied by him into temperature groups is itself a variable, it is questionable whether varieties generally are as susceptible to small difference in the amount of heat received during the growing season as he considered them to be. The problem can be successfully solved only under conditions which permit accurate measurement of each of the climatic factors of the environment and control of soil and cultural treatment.

#### PURPOSE AND METHOD[OF THE PRESENT WORK

In the work here reported an attempt has been made to obtain an experimental measure of the effect upon the chemical composition of apples of the differences in climatic conditions occurring over a series of years, the factors of soil and cultural treatment being constant. This has been done by bringing together, under controlled conditions, a large number of varieties of widely diverse origin and degree of adaptation to the local climatic conditions, growing them over a series of years, and making a study of the physical and chemical character of the fruit, with simultaneous study and analysis of the climatic conditions. It was believed that if varieties have only a limited capacity of adjustment to environment, those which found it impossible to adjust themselves to the climatic conditions would manifest the fact by constant departure from their normal composition and character as grown under conditions to which they are adjusted. The variations in seasonal conditions which occur in the

course of a series of years should also make for better development of one or another group according as the particular season simulates that of a warmer or cooler latitude. If the variations in seasonal conditions were sufficiently wide and the experiments were continued long enough to permit checking of the results against themselves, it was believed that it would be possible to obtain a valuation of the climatic factors in terms of the composition of the fruit, without modifications due to differences in soils, cultural treatment, or other factors not controllable in studies of material collected from widely separated sources.

In the course of a systematic study of the effects of seasonal variations in climatic conditions upon the chemical composition of apples (1, 2), approximately 100 of the varieties assigned by Shaw to various optimum temperature groups have been employed. These were growing together in the apple variety collection of the Bureau of Plant Industry, United States Department of Agriculture, at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. For some 70 of these, analyses of the expressed juices of the fruit were regularly made each year for all the varieties bearing crops of normal size, over a period of six years. For about 30 additional varieties analyses were made in two or three years of the period, but not necessarily in consecutive years or in every year in which the trees were in fruit. All of the temperature groups are represented, most of the more important ones by a majority of their members.

The conditions under which the samples were taken have been stated in detail elsewhere (1) and will be only briefly summarized here. The trees, two of each variety, were 13 to 16 years of age at the time the first samples of fruit were taken in 1920. They were in all cases free of discoverable disease, making normal growth, and in condition considered typical for healthy trees of the variety. None showed evidence of lack of adjustment to the local conditions. The samples of fruit employed in every case consisted of 1 to 5 bushels of the unsorted tree-run fruit of the mixed crop of the two trees of the variety. The fruit was held in cellar storage or, in a few cases, in cold storage for a short time, followed by transfer to cellar storage until it had attained proper condition for pressing, all the samples being brought to the same degree of ripeness. Pressing was so carried out as to obtain a uniform degree of extraction of the juice, and portions for analysis were taken only after thorough stirring of the bulk sample to insure uniformity. Analyses were always begun as soon as pressing was completed and were always finished on the same day.

#### ANALYTICAL RESULTS

In the tabulation of the analytical data (Table 1) the varieties have been arranged by optimum temperature groups, beginning with the lowest or 52° F. group and ending with the highest or 67° group.

The average mean temperature at Washington for the seven months, March to September, inclusive, which is the period employed by Shaw in computing mean summer temperature, is 64.5° F. For



the six years during which the analyses were being made, the variation in mean summer temperature was from 3.7 degrees above the 50-year average to 1.6 degrees below it, or a range of 5.3 degrees. For the various years the means were as follows: 1920, 64° F.; 1921, 68.2°; 1922, 65.9°; 1923, 65.4°; 1924, 62.9°; 1925, 66.3°.

On the basis of the conclusions reached by Shaw—namely, that temperature is the dominant factor in determining quality, that every apple variety has an optimum temperature at which it attains its best development, and that when grown at temperatures as much as 2 degrees above or below the optimum it undergoes definite modifications, physical and chemical, which result in lowering of dessert and keeping quality—certain general tendencies should be at once apparent upon examination of the analytical data. Some of these may be stated at this point.

1. Since the mean summer temperature at the Arlington Experiment Farm approximates the optimum for the high-temperature groups, it should follow that the varieties of the 64° to 67° F. groups should attain their best development year after year without marked departures therefrom.

2. Since the members of the 52° to 63° groups are growing at the Arlington farm under summer temperatures higher than their various optima by amounts ranging from 2 to 13 degrees, they should constantly show evidence of injurious effects of the excessive temperatures in the form of modification in composition and keeping quality of the fruit.

3. Such modifications due to excessive temperature should become evident in the 62° group, of which Akin, Grimes Golden, and York Imperial are representatives, and should progressively increase through the groups to a maximum in Baxter, Beitigheimer, Yellow Transparent, and Okabena, of the 53° and 52° groups.

4. The actual summer means during the period show considerable variation from the average, dropping to the 63° level in 1924 and rising above the optimum for the highest temperature group in 1921. The low temperature of 1924 should be productive of retarded maturity and lowered dessert and keeping quality in the 64° to 67° groups, while the quality of the 61° to 63° groups should be improved by reason of the nearer approach to their optimum temperature conditions. In 1921 the excessively high seasonal average of 68.2° F. should result in an acceleration of maturity and decline in quality which should be especially evident in the 63° to 67° groups when the results are compared with those of cooler years. In some degree, at least, the extremes occurring in these two years should be productive of detectable effects opposite in character throughout a considerable number of groups.

5. All the effects just stated should be most marked in the late-maturing long-season varieties and less evident in early-maturing varieties.

TABLE 1.—Analytical data on apple varieties (arranged by optimum temperature groups)

Temperature (°F.) group and variety	Date picked	Date analyzed	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar	Acid as malic	Total astrin- gency	Tan- nin	Non- tan- nins	Total solids
52 group:										
Okabena.....	Aug. 14, 1920	Sept. 19	6.40	1.84	8.24	0.988	0.0802	0.0334	0.0468	-----
	Aug. 29, 1924	Sept. 2	5.80	2.32	8.12	.477	.0891	.0326	.0565	-----
53 group:										
Aretic.....	Sept. 28, 1922	Dec. 8	8.26	2.15	10.41	.439	.1155	.0358	.0797	-----
	Sept. 16, 1923	Nov. 15	9.13	4.84	13.97	.608	.1648	.0622	.1026	16.76
	Sept. 21, 1924	Oct. 2	6.72	3.38	10.10	.525	.1660	.0760	.0900	-----
	Sept. 10, 1925	Sept. 26	8.62	1.84	10.46	.360	.1265	.0518	.0747	13.33
	Sept. 13, 1923	Sept. 27	6.94	3.87	10.81	.634	.1010	.0308	.0702	13.06
Batter.....	Sept. 13, 1924	Oct. 7	4.80	2.24	7.04	.340	.0780	.0257	.0523	-----
	Sept. 17, 1925	Sept. 24	7.02	2.30	9.32	.352	.0932	.0414	.0518	11.81
Bietigheimer.....	Aug. 11, 1920	Sept. 21	5.90	1.77	7.67	.762	.0985	.0447	.0538	9.48
	Aug. 1, 1922	Aug. 2	7.86	1.30	9.16	.820	.1126	.0166	.0960	10.92
	Aug. 12, 1921	Sept. 6	8.16	3.13	11.29	.427	-----	-----	-----	-----
Bismarck.....	Sept. 8, 1922	Dec. 14	7.86	2.02	9.88	.688	.0826	.0227	.0599	10.87
	Oct. 8, 1924	Oct. 15	8.63	.41	9.04	.630	.1435	.0550	.0885	11.51
	Aug. 17, 1920	Sept. 2	8.40	1.78	10.18	.752	-----	-----	-----	-----
Dudley.....	July 13, 1922	July 28	6.21	3.15	9.36	1.093	.1369	.0245	.1124	11.98
	Aug. 4, 1923	Aug. 13	5.90	2.30	8.20	.762	.1223	.0478	.0745	10.58
	Sept. 5, 1924	Sept. 12	5.08	2.58	7.66	.545	.0791	.0351	.0440	-----
Tetofski.....	June 25, 1922	June 30	5.10	2.38	7.48	.614	.1451	.0511	.0940	8.68
	July 1, 1923	July 6	5.18	3.50	8.68	.721	.1436	.0706	.0730	9.93
	June 26, 1922	June 30	6.34	1.56	7.90	.680	.1942	.1125	.0817	9.24
Yellow Transparent..	July 9, 1923	July 18	7.35	2.07	9.42	1.400	.2270	.0876	.1394	12.17
	July 23, 1924	July 30	5.31	.77	6.08	.439	.1360	.0380	.0980	9.69
	July 11, 1925	July 17	5.92	1.61	7.53	.486	.0753	.0255	.0498	-----
54 group:										
Fameuse.....	Sept. 12, 1920	Sept. 27	10.00	1.26	11.26	.420	-----	-----	-----	12.71
	Sept. 27, 1923	Oct. 4	8.98	3.05	12.03	.580	.0960	.0308	.0652	15.45
	Sept. 15, 1924	Sept. 25	7.12	2.32	9.44	.454	.0900	.0362	.0538	-----
	Sept. 15, 1925	Sept. 23	8.80	2.48	11.28	.325	.0996	.0454	.0542	13.95
Gideon.....	Aug. 18, 1920	Sept. 16	8.94	1.08	10.02	.678	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 6	7.62	2.55	10.17	.456	.0956	.0477	.0479	12.31
	Sept. 5, 1924	Sept. 19	7.46	1.02	8.48	.362	.1080	.0605	.0475	-----
Milwaukee.....	Aug. 30, 1922	do.	7.54	1.24	8.78	.654	.0712	.0304	.0408	11.04
	Sept. 24, 1924	Oct. 10	6.26	.94	7.20	.805	.1150	.0395	.0755	-----
Red Astrachan.....	June 26, 1922	June 30	6.82	2.76	9.58	.960	.2095	.0787	.1308	11.63
	July 14, 1923	July 18	7.46	3.21	10.67	1.380	.2550	.1180	.1370	13.48
	Nov. 1, 1920	Nov. 20	8.86	2.02	10.88	.610	-----	-----	-----	12.87
	Sept. 13, 1921	Oct. 16	8.64	2.77	11.41	.571	-----	-----	-----	-----
Walbridge.....	Sept. 26, 1922	Sept. 29	8.10	1.52	9.62	.680	.1034	.0501	.0443	13.14
	Oct. 20, 1923	Oct. 25	10.40	2.96	13.36	.535	.1052	.0452	.0600	15.16
	Sept. 13, 1924	Oct. 9	8.90	1.24	10.14	.660	.1225	.0445	.0780	-----
	Sept. 23, 1925	Nov. 3	6.77	1.23	8.00	.412	.0977	.0432	.0545	10.87
	Sept. 1, 1920	Oct. 2	7.39	1.38	8.77	.520	-----	-----	-----	-----
	Aug. 19, 1922	Aug. 25	5.68	1.96	7.64	.786	.0766	.0235	.0531	10.18
Wolf River.....	Sept. 27, 1923	Sept. 29	7.87	1.81	9.68	.740	.0940	.0352	.0588	13.22
	Sept. 15, 1924	Sept. 20	5.76	1.80	7.62	.559	.0765	.0342	.0423	-----
	Sept. 16, 1925	Sept. 23	5.70	3.01	8.71	.560	.0750	.0333	.0417	11.17
55 group:										
Blenheim.....	Sept. 13, 1922	Dec. 12	7.05	4.65	11.70	.646	.0650	.0237	.0413	13.02
	Sept. 13, 1923	Nov. 21	7.10	7.01	14.11	.870	.1455	.0721	.0734	16.00
	Oct. 8, 1924	Oct. 21	6.64	4.58	11.22	.590	.0945	.0423	.0522	-----
Cox Orange.....	Sept. 26, 1922	Nov. 2	7.27	3.82	11.09	.401	.1203	.0539	.0661	-----
	Sept. 14, 1923	Sept. 27	7.22	4.78	12.00	.690	.1107	.0397	.0710	13.64
	Aug. 4, 1923	Aug. 13	6.82	1.79	8.61	.605	.1035	.0436	.0590	10.94
Gravenstein.....	July 27, 1925	Aug. 16	5.73	1.46	7.19	.582	.1326	.0564	.0762	-----
	Oct. 13, 1922	Oct. 20	8.58	2.95	11.53	.558	.1088	.0495	.0593	13.71
	Oct. 20, 1923	Oct. 22	8.36	5.26	13.62	.690	.1112	.0477	.0635	16.31
Mann.....	Nov. 4, 1924	Dec. 1	8.92	1.48	10.40	.580	.1090	.0169	.0921	-----
	Sept. 25, 1925	Nov. 16	10.44	2.98	13.42	.417	.1330	.0686	.0644	16.75
	Aug. 7, 1922	Aug. 8	6.92	.58	7.50	.983	.1349	.0287	.1062	10.07
McMahon.....	Aug. 15, 1923	Aug. 17	6.36	1.88	8.24	1.016	.0870	.0378	.0492	9.81
	Aug. 29, 1924	Sept. 3	6.35	1.35	7.70	.752	.0637	.0214	.0723	-----
	Aug. 1, 1925	Sept. 8	6.06	2.68	8.74	.508	.0876	.0328	.0548	10.94
	Oct. 1, 1920	Nov. 1	8.48	2.21	10.69	.500	-----	-----	-----	13.41
	Sept. 30, 1921	Oct. 24	8.69	2.51	11.20	.678	-----	-----	-----	-----
Northwestern Green- ing.....	Oct. 7, 1922	Oct. 12	7.84	2.56	10.40	.368	.0938	.0430	.0508	13.45
	Sept. 28, 1923	Nov. 12	8.36	2.21	10.57	.420	.1174	.0617	.0557	14.28
	Nov. 4, 1924	Nov. 15	8.12	1.09	9.21	.339	.0878	.0278	.0600	-----
	Oct. 5, 1925	Nov. 24	9.16	1.75	10.91	.378	.0735	.0177	.0558	12.84
Patten.....	Aug. 24, 1920	Sept. 17	6.40	.92	7.32	.678	.0513	.0113	.0400	9.64
	Aug. 15, 1922	Aug. 18	7.55	1.47	9.02	.964	.1226	.0347	.0379	-----
	Sept. 24, 1924	Oct. 2	5.38	1.58	6.96	.630	.1250	.0578	.0672	-----

TABLE 1.—*Analytical data on apple varieties (arranged by optimum temperature groups)—Continued*

Temperature (°F.) group and variety	Date picked	Date analyzed	Constituents (per cent)							
			Re- ducing sugar	Su- crose as in- vert sugar	Total sugar	Acid as malic	Total astrin- gency	Tan- nin	Non- tan- nins	Total solids
55 group—Continued.										
Salome.....	Sept. 23, 1922	Dec. 9	8.80	1.60	10.40	0.654	0.0466	0.0090	0.0376	13.25
	Oct. 20, 1923	Oct. 22	8.41	4.79	13.20	.667	.0831	.0299	.0532	14.08
	Oct. 8, 1924	Oct. 10	7.58	2.98	10.56	.773	.0970	.0190	.0780	-----
	Sept. 15, 1925	do	10.06	2.15	12.21	.482	.0927	.0285	.0642	-----
Scott Winter.....	Sept. 23, 1922	Sept. 26	6.73	3.39	10.12	1.030	.0825	.0434	.0391	12.36
	Oct. 4, 1923	Nov. 5	6.56	3.62	10.18	.930	.1029	.0370	.0659	12.16
	Sept. 27, 1924	Oct. 4	10.26	6.30	16.56	.976	.1030	.0403	.0627	18.33
Sept. 22, 1925	Oct. 16	7.22	2.25	9.47	.712	.1108	.0398	.0710	12.26	
56 group:										
Baldwin.....	Oct. 11, 1920	Nov. 2	8.72	1.39	10.11	.630	-----	-----	-----	12.96
	Sept. 2, 1921	Nov. 6	9.00	3.77	12.77	.595	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 5	7.36	3.17	10.53	.571	.0885	.0324	.0561	-----
	Oct. 26, 1923	Oct. 31	9.40	5.56	14.96	.635	.1346	.0465	.0881	16.02
	Oct. 20, 1924	Oct. 30	7.24	4.24	11.48	.430	.1237	.0259	.0978	-----
Early Harvest.....	Sept. 16, 1925	Oct. 6	6.76	3.43	10.19	.424	.0953	.0343	.0610	-----
	July 9, 1922	July 10	4.60	3.32	7.92	.901	.1676	.0971	.0705	10.39
	July 16, 1923	July 23	4.68	2.16	6.84	.552	.1221	.0528	.0693	-----
Golden Russet.....	Oct. 8, 1920	Oct. 26	10.28	.89	10.67	.478	.0900	.0332	.0568	12.39
	Sept. 23, 1922	Nov. 17	11.01	1.33	12.34	.476	.0890	.0223	.0637	-----
	Sept. 28, 1923	Oct. 5	9.43	3.55	12.98	.605	.0981	.0386	.0575	15.46
	Oct. 8, 1924	Oct. 21	9.00	2.82	11.82	.447	.0800	.0303	.0497	-----
McIntosh.....	Sept. 1, 1920	Sept. 16	8.13	1.66	9.79	.423	-----	-----	-----	-----
	Sept. 1, 1922	Sept. 5	8.03	1.39	9.42	.566	.0966	.0518	.0448	11.36
	Sept. 5, 1923	Sept. 13	8.00	3.34	11.34	.646	.1248	.0616	.0632	13.44
	Sept. 13, 1924	Sept. 25	7.04	3.36	10.40	.419	.0717	.0294	.0428	-----
	Aug. 21, 1925	Sept. 8	6.66	2.75	9.41	.371	.1040	.0398	.0642	11.84
Northern Spy.....	Sept. 28, 1922	Nov. 3	8.12	2.86	10.98	.393	.1239	.0425	.0814	13.09
	Sept. 29, 1924	Oct. 8	6.54	3.16	9.70	.567	.1055	.0403	.0652	-----
	Sept. 16, 1925	Sept. 22	8.06	3.32	11.38	.495	.1340	.0656	.0684	13.61
	Sept. 21, 1922	Sept. 23	5.72	1.74	7.46	.667	.0738	.0373	.0365	8.66
Ontario.....	Oct. 1, 1924	Oct. 6	5.74	1.28	7.02	.805	.1400	.0757	.0643	-----
	Oct. 15, 1920	Nov. 11	6.72	3.20	9.92	.386	-----	-----	-----	-----
	Sept. 23, 1922	Nov. 3	7.36	2.78	10.14	.456	.1071	.0354	.0717	12.71
Sutton.....	Sept. 23, 1923	Oct. 5	7.58	4.92	12.50	.615	.0815	.0267	.0548	13.37
	Sept. 24, 1924	Oct. 8	11.94	1.76	13.70	.585	.0960	.0368	.0592	-----
	Oct. 1, 1925	Oct. 6	7.18	5.38	12.56	.384	.1170	.0470	.0700	15.52
Tolman Sweet.....	Oct. 22, 1923	Nov. 2	7.78	5.63	13.41	.222	.0900	.0387	.0513	14.26
	Oct. 8, 1924	Oct. 16	6.31	4.19	10.50	.170	.0865	.0410	.0455	-----
	Sept. 16, 1920	Oct. 16	7.94	2.06	10.00	.537	-----	-----	-----	-----
Tompkins King....	Sept. 23, 1922	Dec. 11	8.71	1.74	10.45	.542	.0984	.0501	.0483	13.07
	Sept. 28, 1923	Oct. 8	9.48	3.25	12.73	.410	.1123	.0428	.0695	15.93
	Sept. 24, 1924	Oct. 3	7.23	2.75	9.98	.423	.1030	.0342	.0688	-----
	Sept. 15, 1925	Oct. 19	7.26	4.07	11.33	.350	.1075	.0407	.0688	13.65
	Aug. 11, 1922	Aug. 14	7.20	1.37	8.57	.709	.0848	.0061	.0787	10.37
Wealthy.....	Aug. 23, 1923	Sept. 4	7.33	2.87	10.20	.622	.0796	.0203	.0593	11.79
	Aug. 29, 1924	Sept. 3	7.38	1.75	9.13	.665	.1140	.0575	.0565	11.20
	Sept. 6, 1920	Oct. 17	9.22	2.30	11.52	.482	-----	-----	-----	-----
Westfield.....	Oct. 3, 1922	Oct. 7	7.33	3.03	10.36	.466	.1113	.0561	.0552	12.96
	Oct. 26, 1923	Nov. 2	8.00	5.87	13.87	.525	.1157	.0429	.0728	15.42
	Oct. 14, 1924	Oct. 17	7.86	2.34	10.20	.437	.0840	.0230	.0610	-----
	Oct. 7, 1925	Oct. 16	7.24	4.94	12.18	.415	.1200	.0390	.0810	14.95
57 group:										
Babbitt.....	Aug. 29, 1922	Sept. 14	7.04	2.96	10.00	1.182	.1095	.0348	.0747	12.70
	Sept. 27, 1923	Sept. 29	7.44	3.26	10.70	1.130	.0957	.0317	.0640	12.98
	Sept. 13, 1924	Oct. 8	7.64	2.92	10.56	1.112	.1185	.0370	.0815	-----
	Sept. 17, 1925	Oct. 7	8.44	2.01	10.45	.910	.1002	.0337	.0665	13.54
	Sept. 14, 1920	Sept. 27	6.86	2.10	8.96	.830	-----	-----	-----	-----
Boiken.....	Sept. 23, 1922	Oct. 19	6.50	3.98	10.48	.811	.0672	.0274	.0398	11.08
	Sept. 19, 1923	Nov. 30	7.04	3.23	10.27	.589	.0762	.0335	.0427	11.96
	Oct. 8, 1924	Oct. 20	7.00	2.43	9.43	.784	.0635	.0301	.0394	-----
	Sept. 3, 1920	Sept. 27	9.68	1.52	11.20	.595	-----	-----	-----	-----
	Aug. 25, 1921	do	9.02	3.55	12.57	.480	-----	-----	-----	-----
Hubbardston.....	Sept. 18, 1922	Dec. 11	8.22	2.54	10.76	.598	.0949	.0422	.0527	13.47
	Sept. 27, 1923	Oct. 5	7.18	3.76	10.94	.410	.0746	.0241	.0505	12.75
	Oct. 1, 1925	Oct. 20	6.94	2.95	9.89	.168	.0878	.0377	.0501	11.74
	Aug. 14, 1920	Sept. 21	8.12	1.09	9.21	.510	-----	-----	-----	11.25
	Aug. 1, 1921	Sept. 5	7.96	2.62	10.58	.607	-----	-----	-----	-----
Jefferis.....	July 27, 1922	July 31	7.52	1.04	8.56	.808	.1481	.0418	.1063	10.69
	Aug. 23, 1923	Aug. 30	8.15	5.21	13.36	.642	.1165	.0568	.0597	14.87
	Aug. 29, 1924	Sept. 6	7.98	3.30	11.28	.342	.1050	.0735	.0315	-----
	Aug. 8, 1925	Aug. 15	8.24	3.53	11.77	.508	.1010	.0497	.0513	-----
	Sept. 8, 1921	Oct. 6	6.54	2.02	8.56	.216	.1196	.0626	.0570	10.89
Lady Sweet.....	Sept. 8, 1923	Nov. 30	7.49	2.82	10.31	.196	.1031	.0431	.0600	12.00

TABLE 1.—Analytical data on apple varieties (arranged by optimum temperature groups)—Continued

Temperature (°F.) group and variety	Date picked	Date analyzed	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar	Acid as malic	Total astringency	Tannin	Non-tannins	Total solids
57 group—Continued.										
Melon.....	Sept. 25, 1920	Oct. 6	8.98	1.45	10.43	0.354	0.1032	0.0237	0.0795	11.91
	Sept. 1, 1922	Sept. 14	7.25	3.23	10.48	.505	.1190	.0547	.0643	14.03
	Oct. 20, 1923	Oct. 26	7.70	1.74	9.44	.277	.0858	.0403	.0455	10.14
	Oct. 1, 1925	Oct. 12	9.14	1.10	10.24	.195	.1246	.0495	.0751	-----
Monmouth.....	Oct. 7, 1920	Oct. 30	7.74	2.02	9.76	.602	-----	-----	-----	11.85
	Sept. 18, 1922	Sept. 23	7.60	1.99	9.59	.543	.0677	.0286	.0391	12.68
	Oct. 20, 1923	Nov. 6	7.74	3.03	10.77	.386	.0857	.0300	.0557	13.24
	Oct. 21, 1924	do.....	7.06	.96	8.02	.413	.0955	.0287	.0668	-----
Primate.....	Oct. 5, 1925	Nov. 16	9.32	1.75	11.07	.300	.0985	.0403	.0582	12.91
	July 20, 1922	July 21	6.22	3.52	9.74	.478	.1287	.0310	.0977	10.94
	July 20, 1923	July 23	7.34	2.58	9.92	.890	.1424	.0887	.0537	11.32
	Aug. 6, 1924	Aug. 11	7.26	.38	7.64	1.385	.0740	.0318	.0422	-----
Pumpkin Sweet.....	Sept. 27, 1923	Oct. 6	8.82	3.82	12.64	.231	.1036	.0489	.0547	14.34
	Sept. 13, 1924	Oct. 4	7.44	2.86	10.30	.150	.1330	.0505	.0825	-----
Roxbury Russet.....	Oct. 4, 1920	Nov. 2	9.13	2.75	11.88	.537	-----	-----	-----	13.03
	Oct. 4, 1923	Nov. 5	6.29	5.98	12.27	.614	.1130	.0471	.0659	16.84
	Oct. 8, 1924	Oct. 22	6.16	3.94	10.10	.532	.0945	.0373	.0572	-----
	Oct. 1, 1925	Oct. 14	8.60	4.60	13.20	.502	.1200	.0365	.0835	16.41
Williams.....	July 25, 1923	July 30	6.67	4.10	10.77	.551	.1420	.0695	.0725	12.32
	July 29, 1924	Aug. 6	6.67	2.15	8.82	.296	.1275	.0606	.0669	-----
July 21, 1925	July 25	6.71	1.53	8.24	.387	.0856	.0311	.0545	-----	
58 group:										
Early Joe.....	July 20, 1922	July 21	6.41	2.19	8.60	.424	.1390	.0583	.0807	9.72
	Aug. 10, 1924	Aug. 11	7.44	.72	8.16	.352	.1742	.0792	.0950	-----
Early Strawberry.....	July 21, 1921	Aug. 3	5.60	2.30	7.90	.644	-----	-----	-----	10.20
	July 25, 1923	July 30	6.18	3.18	9.36	.733	.1375	.0735	.0640	11.20
Ewalt.....	Aug. 29, 1922	Sept. 16	4.85	2.72	7.57	.595	.1086	.0565	.0521	10.03
	Sept. 10, 1924	Sept. 26	5.79	1.83	7.62	.707	.1250	.0586	.0664	-----
Golden Sweet.....	July 31, 1920	Aug. 6	8.86	1.12	9.98	.144	.1280	.0460	.0820	11.40
	July 25, 1922	July 26	8.37	1.20	9.57	.276	.1768	.0521	.1247	-----
	Aug. 2, 1923	Aug. 6	6.09	3.43	9.52	.200	.1880	.0845	.1035	10.72
Lowell.....	Aug. 14, 1920	Aug. 21	6.34	3.14	9.48	.570	.1260	.0670	.0590	-----
	Aug. 16, 1923	Aug. 24	6.60	3.08	9.68	.510	.1370	.0710	.0660	11.07
Mother.....	Sept. 1, 1920	Sept. 19	9.46	.57	10.03	.413	-----	-----	-----	-----
	Aug. 30, 1922	Sept. 15	6.89	4.15	11.04	.347	.1034	.0313	.0721	13.21
	Sept. 8, 1923	Sept. 22	6.86	4.48	11.34	.422	.1095	.0420	.0675	12.50
	Aug. 29, 1924	Sept. 15	6.97	4.37	11.34	.487	.1215	.0717	.0498	-----
Red June.....	Sept. 14, 1925	Sept. 22	8.24	4.02	12.26	.267	.1242	.0742	.0500	14.08
	July 15, 1922	July 17	6.01	1.84	7.85	.551	.1584	.0603	.0981	10.56
	July 20, 1923	July 23	6.06	2.93	8.99	.686	.1373	.0713	.0660	10.30
	July 28, 1924	Aug. 13	6.54	2.43	8.97	.350	.1180	.0390	.0790	-----
Swaar.....	Oct. 8, 1920	Oct. 30	10.13	3.77	13.90	.612	-----	-----	-----	14.66
	Sept. 23, 1922	Oct. 31	8.07	3.71	11.78	.598	.1115	.0398	.0717	14.97
	Sept. 28, 1923	Oct. 8	8.14	4.64	12.78	.587	.0832	.0276	.0556	15.12
	Oct. 11, 1924	Oct. 22	9.03	3.64	12.67	.762	.1280	.0525	.0755	-----
Twenty Ounce.....	Oct. 1, 1925	Oct. 23	8.06	4.42	12.48	.437	.0705	.0135	.0670	14.92
	Sept. 16, 1923	Nov. 28	8.28	3.57	11.85	.572	.0839	.0394	.0445	14.70
	Sept. 13, 1924	Sept. 18	6.15	2.95	9.10	.576	.0833	.0368	.0465	-----
	Aug. 22, 1921	Sept. 19	7.40	3.45	10.85	.293	-----	-----	-----	-----
Winter Banana.....	Sept. 6, 1922	Sept. 13	6.63	4.10	10.73	.317	.1181	.0634	.0547	12.42
	Sept. 5, 1923	do.....	8.20	2.33	10.53	.371	.1530	.0540	.0990	12.02
	Sept. 18, 1925	Oct. 5	9.18	1.98	10.84	.182	.1390	.0570	.0820	13.18
59 group:										
Benoni.....	July 19, 1922	Aug. 9	6.04	2.80	8.84	.426	.1308	.0429	.0879	9.62
	July 30, 1923	July 30	6.04	4.30	10.34	.655	.1485	.0700	.0785	11.62
Delicious.....	Sept. 25, 1920	Oct. 6	9.55	1.05	10.60	.254	-----	-----	-----	-----
	Sept. 21, 1921	Oct. 18	7.39	4.30	11.69	.313	-----	-----	-----	-----
	Oct. 7, 1922	Oct. 11	7.28	2.82	10.10	.279	.0868	.0377	.0491	12.46
	Oct. 14, 1924	Nov. 4	8.24	1.98	10.22	.216	.0760	.0322	.0438	-----
Esopus Spitzenburg.....	Sept. 23, 1922	Nov. 16	8.41	2.23	10.64	.451	.0700	.0205	.0495	14.13
	Oct. 4, 1924	Oct. 15	7.86	4.68	12.54	.778	.0918	.0300	.0618	-----
	Aug. 19, 1922	Aug. 21	8.71	2.67	11.38	1.053	.1191	.0255	.0936	13.26
	Sept. 16, 1923	Dec. 6	7.26	2.15	9.41	.445	.0852	.0272	.0580	14.30
Haas.....	Sept. 5, 1924	Sept. 19	7.05	1.57	8.62	.583	.1170	.0410	.0760	-----
	Sept. 14, 1925	Sept. 22	7.72	3.11	10.83	.477	.1320	.0500	.0820	13.84
	Oct. 21, 1922	Nov. 3	8.32	2.77	11.09	.380	.0938	.0336	.0602	13.02
	Oct. 16, 1923	Nov. 27	7.20	6.68	12.88	.542	.1077	.0431	.0646	15.34
King David.....	Aug. 30, 1921	Nov. 1	7.79	2.91	10.70	.372	-----	-----	-----	-----
	Sept. 23, 1922	Nov. 17	7.79	4.52	12.31	.561	.0749	.0263	.0486	15.56
	Sept. 19, 1923	Nov. 16	9.28	4.42	13.70	.651	.0692	.0092	.0600	14.48
	Sept. 6, 1921	Oct. 8	8.34	4.44	10.78	.481	.0893	.0253	.0640	-----
Kinnard.....	Sept. 28, 1923	Nov. 5	7.82	3.94	11.76	.435	.1080	.0439	.0641	13.66
	Oct. 8, 1924	Oct. 22	6.64	3.38	10.02	.470	.1045	.0473	.0572	-----
	Oct. 15, 1925	Oct. 31	7.54	2.09	9.63	.314	.0899	.0302	.0597	12.19

TABLE 1.—Analytical data on apple varieties (arranged by optimum temperature groups)—Continued

Temperature (°F.) group and variety	Date picked	Date analyzed	Constituents (per cent)							
			Reducing sugar	Sucrose as invert sugar	Total sugar	Acid as malic	Total astrin-gency	Tan-nin	Non-tan-nins	Total solids
59 group—Continued.										
Red Canada.....	{Oct. 1, 1920	Oct. 6	7.86	2.57	10.43	0.316	0.0700	0.0210	0.0490	12.09
	{Sept. 27, 1923	do.....	9.59	3.97	13.56	.422	.0832	.0430	.0402	15.57
Wagener.....	{Sept. 16, 1923	Dec. 4	6.24	3.88	10.12	.567	.0795	.0290	.0505	13.12
	{Oct. 1, 1924	Oct. 7	6.06	3.48	9.54	.570	.0917	.0232	.0685	-----
60 group:										
Domine.....	{Oct. 7, 1922	Oct. 11	6.78	3.15	9.93	.426	.0947	.0711	.0236	12.83
	{Oct. 8, 1923	Nov. 19	8.55	4.43	12.98	.514	.1110	.0606	.0504	12.10
	{Oct. 11, 1924	Oct. 21	7.13	2.71	9.84	.445	.0635	.0267	.0368	-----
	{Sept. 17, 1925	Oct. 8	8.70	3.19	11.89	.372	.0920	.0378	.0542	14.84
	{Sept. 18, 1920	Oct. 20	9.63	1.14	10.77	.374	-----	-----	-----	12.16
	{Sept. 18, 1922	Oct. 31	9.26	-----	9.26	.221	.1124	.0355	.0769	12.23
Fallowater.....	{Sept. 27, 1923	Oct. 4	8.61	3.42	12.03	.418	.0780	.0162	.0518	15.42
	{Oct. 8, 1924	Oct. 11	7.53	2.24	9.77	.250	.0832	.0316	.0516	-----
	{July 26, 1923	July 28	6.23	1.26	7.49	1.104	.1359	.0123	.1266	9.86
	{Sept. 3, 1923	Sept. 11	6.49	1.81	8.30	.454	.0930	.0430	.0500	8.86
Hagloe Crab.....	{Sept. 5, 1924	Sept. 12	6.24	.84	7.08	.494	.1025	.0495	.0530	-----
	{Aug. 21, 1925	Sept. 3	5.58	2.08	7.66	.449	.0965	.0425	.0540	10.94
	{Oct. 5, 1921	Oct. 26	7.86	3.05	10.91	.620	-----	-----	-----	-----
	{Oct. 3, 1922	Oct. 11	7.49	2.60	10.09	.507	.1078	.0499	.0579	13.01
McAfee.....	{Oct. 13, 1923	Oct. 26	9.34	2.66	12.00	.394	.1035	.0428	.0607	13.60
	{Oct. 21, 1924	Nov. 13	8.24	1.34	9.58	.375	.1132	.0422	.0710	-----
	{Oct. 15, 1925	Nov. 16	8.56	1.68	10.24	.235	.1210	.0480	.0730	11.95
	{Oct. 3, 1922	Oct. 5	7.22	2.96	10.18	.510	.0771	.0228	.0543	-----
Newtown Spitzen-burg.....	{Oct. 4, 1923	Nov. 19	7.44	3.87	11.31	.367	.0852	.0442	.0410	14.02
	{Oct. 18, 1924	Nov. 25	6.31	1.61	7.92	.325	.0702	.0314	.0388	-----
	{Sept. 25, 1925	Oct. 17	7.02	2.98	10.00	.273	.0760	.0302	.0458	11.88
	{Sept. 2, 1920	Sept. 23	8.90	2.01	10.91	.388	.0920	.0352	.0568	12.88
Rambo.....	{Oct. 20, 1923	Oct. 26	9.48	4.06	13.54	.402	.1002	.0445	.0557	14.84
	{Sept. 14, 1924	Sept. 19	6.76	1.70	8.46	.398	.0795	.0070	.0725	-----
	{Oct. 7, 1922	Oct. 9	6.62	3.79	10.41	.360	.0877	.0264	.0613	11.99
	{Oct. 9, 1923	Oct. 18	6.88	5.23	12.11	.392	.0720	.0164	.0556	13.41
Rome Beauty.....	{Nov. 4, 1924	Nov. 29	6.20	2.02	8.22	.264	.0836	.0146	.0690	11.62
	{Sept. 23, 1925	Nov. 3	5.99	2.73	8.72	.273	.0963	.0329	.0664	11.28
	{Sept. 3, 1921	Sept. 26	9.07	3.81	12.88	.672	-----	-----	-----	-----
	{Sept. 28, 1922	Oct. 13	6.77	4.12	10.89	.553	.0747	.0204	.0543	12.09
Smokehouse.....	{Oct. 4, 1923	Nov. 14	9.02	4.06	13.08	.559	.1200	.0472	.0728	15.70
	{Sept. 13, 1924	Oct. 8	7.00	1.80	8.80	.440	.1020	.0412	.0608	11.20
	{Oct. 3, 1925	Oct. 22	9.60	2.35	11.95	.378	.1225	.0457	.0768	15.12
	{Oct. 3, 1922	Oct. 18	6.84	4.03	10.87	.537	.0926	.0403	.0523	13.92
Yellow Newtown.....	{Oct. 11, 1924	Oct. 21	7.13	3.19	10.32	.593	.0747	.0260	.0487	14.12
61 group:										
Lankford.....	{Sept. 2, 1921	Sept. 13	7.06	4.59	11.65	.504	.0873	.0401	.0472	-----
	{Oct. 11, 1924	Oct. 20	6.83	3.52	10.35	.507	.0925	.0428	.0497	-----
	{Sept. 23, 1922	Sept. 23	6.04	3.23	9.27	.522	.0634	.0348	.0286	11.42
Ortley.....	{Sept. 27, 1923	Sept. 29	8.00	3.24	11.24	.732	.0842	.0342	.0500	13.24
	{Oct. 8, 1924	Oct. 21	7.64	2.29	9.93	.400	.0713	.0318	.0395	-----
Smith Cider.....	{Sept. 23, 1922	Dec. 11	7.74	2.11	9.85	.524	.0879	.0264	.0615	12.89
	{Oct. 15, 1924	Oct. 17	6.68	3.45	10.13	.438	.0928	.0317	.0608	13.24
	{Oct. 9, 1920	Oct. 20	8.68	1.01	9.69	.630	-----	-----	-----	11.12
	{Sept. 11, 1921	Oct. 13	8.40	3.08	11.48	.651	-----	-----	-----	-----
White Pippin.....	{Oct. 13, 1922	Oct. 19	6.34	4.70	11.04	.522	.0717	.0275	.0442	-----
	{Sept. 28, 1923	Nov. 14	6.62	4.77	11.39	.470	.0965	.0430	.0565	13.42
	{Oct. 4, 1924	Oct. 9	6.04	4.02	10.06	.595	.0950	.0308	.0642	-----
	{Oct. 13, 1920	Nov. 2	7.86	2.16	10.02	.636	-----	-----	-----	13.82
	{Sept. 2, 1921	Nov. 6	9.00	3.77	12.77	.595	-----	-----	-----	-----
Yellow Bellflower.....	{Oct. 3, 1922	Oct. 6	7.36	3.17	10.53	.571	.0885	.0324	.0561	-----
	{Oct. 26, 1923	Oct. 31	9.40	5.56	14.96	.635	.1346	.0465	.0881	16.02
	{Sept. 16, 1925	Oct. 6	6.76	3.43	10.19	.424	.0953	.0343	.0610	-----
62 group:										
Akin.....	{Sept. 11, 1921	Oct. 6	8.64	3.31	11.95	.465	-----	-----	-----	-----
	{Oct. 27, 1922	Dec. 5	8.43	2.03	10.46	.432	.1099	.0613	.0486	14.28
	{Oct. 20, 1923	Oct. 25	9.46	3.20	12.66	.391	.0951	.0557	.0394	14.68
	{Oct. 8, 1924	Oct. 17	7.68	1.82	9.50	.384	.0815	.0310	.0505	-----
	{Oct. 15, 1925	Oct. 31	8.48	2.80	11.28	.314	.0772	.0212	.0600	13.56
Grimes Golden.....	{Sept. 18, 1922	Nov. 14	8.44	5.16	13.60	.380	.0911	.0283	.0828	15.02
	{Oct. 14, 1924	Oct. 16	6.34	4.53	10.87	.440	.0797	.0307	.0490	-----
	{Oct. 13, 1922	Nov. 3	6.57	4.13	10.70	.279	.0982	.0292	.0690	11.84
	{Sept. 28, 1923	Oct. 8	7.18	5.14	12.32	.476	.0763	.0216	.0547	13.97
Huntsman.....	{Oct. 8, 1924	Oct. 15	6.98	3.90	10.88	.502	.0832	.0154	.0678	-----
	{Oct. 1, 1925	Oct. 26	6.66	2.48	9.14	.260	.0859	-----	.0550	11.16
	{Sept. 13, 1921	Sept. 26	8.20	3.16	11.36	.442	-----	-----	-----	-----
	{Sept. 23, 1922	Nov. 15	7.46	3.30	10.76	.412	.1208	.0551	.0657	13.46
Ingram.....	{Oct. 8, 1923	Oct. 12	8.00	2.77	10.77	.443	.0727	.0265	.0462	12.49
	{Oct. 7, 1925	Nov. 23	7.92	3.36	11.28	.332	.1205	.0495	.0710	13.31

TABLE 1.—Analytical data on apple varieties (arranged by optimum temperature groups)—Continued

Temperature (°F.) group and variety	Date picked	Date analyzed	Constituents (per cent)							
			Re- duc- ing sugar	Su- cro- se as in- vert sugar	Total sugar	Acid as malic	Total astrin- gency	Tan- nin	Non- tan- nins	Total solids
62 group—Continued.										
Ralls-----	Sept. 2, 1921	Oct. 15	7.39	4.98	12.37	0.680	-----	-----	-----	-----
	Oct. 7, 1922	Oct. 12	7.81	3.07	10.88	.583	0.0789	0.0246	0.0543	14.92
	Oct. 20, 1923	Oct. 26	8.32	3.37	11.69	.420	.0727	.0282	.0445	13.13
	Oct. 14, 1924	Oct. 23	7.62	2.89	10.51	.355	.0640	.0211	.0429	-----
Stark-----	Oct. 10, 1925	Nov. 18	8.76	2.52	11.28	.286	.0950	.0368	.0582	13.31
	Oct. 15, 1922	Oct. 18	7.39	3.30	10.69	.416	.0649	.0412	.0237	12.67
	Oct. 26, 1923	Nov. 2	8.97	4.89	13.86	.481	.0984	.0368	.0616	15.58
	Oct. 21, 1924	Dec. 1	7.72	1.64	9.36	.291	.0885	.0345	.0540	11.29
York Imperial-----	Sept. 13, 1921	Oct. 16	9.00	2.04	11.04	.588	-----	-----	-----	-----
	Sept. 25, 1922	Oct. 12	10.71	1.58	12.29	.491	.0657	.0050	.0607	13.49
	Nov. 1, 1923	Nov. 2	8.36	4.12	12.48	.467	.0770	.0315	.0455	14.80
	Oct. 14, 1924	Oct. 17	7.13	2.43	9.56	.463	.0616	.0136	.0380	-----
63 group:										
Arkansas Black-----	Oct. 12, 1920	Nov. 3	7.42	3.16	10.58	.513	-----	-----	-----	-----
	Sept. 23, 1921	Oct. 16	8.21	3.27	11.48	.625	-----	-----	-----	-----
	Sept. 23, 1922	Dec. 1	8.50	1.84	10.34	.536	.0747	.0273	.0474	12.97
	Sept. 28, 1923	Dec. 3	7.18	2.56	9.74	.439	.0930	.0374	.0556	13.92
Stayman Winesap-----	Oct. 7, 1925	Nov. 24	9.02	2.44	11.46	.360	.0925	.0325	.0600	12.82
	Sept. 10, 1921	Oct. 10	8.16	3.17	11.33	.498	.1165	.0540	.0625	-----
	Oct. 11, 1922	Oct. 21	6.72	3.78	10.50	.507	.0947	.0443	.0504	13.90
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
64 group:										
Ben Davis-----	Oct. 16, 1922	Oct. 30	7.83	2.16	9.99	.385	.1010	.0380	.0630	-----
	Nov. 4, 1924	Dec. 1	7.68	2.38	10.06	.350	.0945	.0329	.0616	-----
	Sept. 10, 1921	Oct. 11	6.25	2.93	9.18	.510	-----	-----	-----	-----
	Oct. 3, 1922	Oct. 10	7.44	2.64	10.08	.406	.1043	.0324	.0719	11.73
Gano-----	Sept. 28, 1923	Dec. 3	7.38	3.53	10.91	.321	.1094	.0500	.0594	11.62
	Oct. 12, 1920	Oct. 30	6.26	1.46	7.72	.617	-----	-----	-----	-----
	Sept. 2, 1921	Oct. 21	8.20	3.43	11.63	.565	-----	-----	-----	-----
	Sept. 29, 1922	Dec. 13	7.77	2.37	10.14	.563	.0729	.0342	.0387	-----
Lawver-----	Sept. 27, 1923	Sept. 29	7.43	3.77	11.20	.694	.0940	.0248	.0692	12.15
	Sept. 15, 1924	Oct. 9	7.74	2.32	10.06	.500	.1260	.0582	.0678	-----
	Oct. 18, 1920	Nov. 13	9.98	2.00	11.98	.510	.0884	.0177	.0707	13.19
	Sept. 10, 1921	Nov. 17	9.30	2.97	12.27	.706	.1055	.0101	.0954	-----
Missouri Pippin-----	Nov. 4, 1924	Nov. 25	6.97	4.93	11.90	.584	.0947	.0329	.0618	-----
	Oct. 13, 1922	Dec. 7	7.51	1.59	9.10	.378	.0629	.0342	.0287	12.01
	Oct. 19, 1923	Nov. 30	8.40	5.63	14.03	.294	.0852	.0221	.0631	14.58
	Oct. 1, 1924	Oct. 4	6.46	3.48	9.94	.482	.1125	.0500	.0565	-----
Oliver Red-----	Sept. 2, 1921	Oct. 3	9.02	2.64	11.66	.507	-----	-----	-----	13.71
	Oct. 26, 1923	Nov. 5	8.10	3.79	11.89	.384	.1280	.0480	.0780	-----
	Nov. 4, 1924	Nov. 22	6.00	2.26	8.26	.332	.0955	.0355	.0600	-----
	Nov. 6, 1925	Nov. 25	10.58	2.30	12.88	.308	.1205	.0601	.0604	13.47
Paragon-----	Oct. 7, 1922	Oct. 10	8.40	4.61	13.01	.520	.0850	.0421	.0429	15.07
	Oct. 19, 1924	Oct. 30	7.26	2.99	10.25	.630	.0962	.0480	.0482	14.00
65 group:										
Beach-----	Oct. 26, 1923	Oct. 31	8.16	4.14	12.30	.398	.0649	.0209	.0440	-----
	Nov. 4, 1924	Nov. 11	7.54	3.60	11.14	.362	.0726	.0152	.0574	-----
	Oct. 15, 1920	Oct. 28	9.50	2.50	12.00	.650	-----	-----	-----	13.51
	Sept. 2, 1921	Sept. 19	8.64	2.43	11.07	.802	-----	-----	-----	-----
Collins-----	Sept. 30, 1922	Nov. 1	7.52	1.54	9.06	.680	.0872	.0265	.0607	11.46
	Oct. 26, 1923	Oct. 31	7.35	5.15	12.50	.740	.1112	.0222	.0890	-----
	Oct. 28, 1924	Nov. 4	6.08	3.34	9.42	.517	.0995	.0295	.0700	-----
	Oct. 7, 1925	Nov. 23	7.82	2.56	10.38	.372	.1058	.0373	.0685	11.57
66 group:										
Buckingham-----	Sept. 23, 1922	Dec. 9	7.65	2.05	9.70	.431	.0448	.0343	.0105	12.19
	Sept. 27, 1923	Oct. 8	6.26	3.39	9.65	.456	.0994	.0463	.0531	11.67
	Oct. 14, 1924	Oct. 17	6.00	1.42	7.42	.356	.0780	.0335	.0445	-----
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
67 group:										
Terry-----	Sept. 2, 1921	Sept. 24	8.16	2.75	10.91	.662	-----	-----	-----	-----
	Oct. 22, 1922	Oct. 24	5.98	4.55	10.53	.786	.1133	.0275	.0858	13.66
	Oct. 26, 1923	Oct. 31	5.90	6.24	12.14	.746	.1232	.0460	.0772	16.40
	Nov. 4, 1924	Nov. 15	5.72	6.39	12.11	.667	.1217	.0422	.0795	-----

The degree to which the behavior of the fruit accords with these expectations based upon Shaw's conclusions is now to be determined by examination of the analytical results. Since the data were collected in the course of a rather comprehensive study of the effect of climatic conditions upon the chemical composition of the fruit, some of the facts established by that study may be briefly summa-

rized, reference being made to the original publication (1) for detailed discussion.

In the study to which reference has been made it was found that the chemical composition of a large number of varieties showed definite response to the climatic conditions, more specifically to the amount of sunshine and heat received during the seven growing months. This correlation was of such a nature that the total sugar content of the crop as a whole was highest in that year which received the largest number of hours of sunshine in the growing season (1923), next highest in the year of maximum temperatures and next-to-maximum hours of sunshine (1921), and lowest in the year of sub-normal temperatures and normal hours of sunshine (1924). The other years of the period 1920-1925 had intermediate positions corresponding to the degree of heat and amount of sunshine received. In other words, the sugar content of the crop of more than 200 varieties increases en masse with increase in the amount of sunshine and temperature over that normally received at Washington and decreases with decrease in the amounts of these factors below the normal. It was further found that there is a definite correlation between total sugar, sucrose, and acidity such that all three are at a maximum or minimum together. Further, there is a correlation between total sugar and astringent substances such that high total sugars are associated with minimum relative astringency, low total sugars with maximum relative astringency. A very large number of varieties of diverse origin were found to behave as one in these respects.

The results as to ranking of the crop of all the varieties for each of the years concerned with respect to total sugar, acidity, and relative astringency are shown in Table 2, together with the mean temperature and amount of sunshine for the season. The latter is expressed as percentage of the normal hours of sunshine for the season.

TABLE 2.—*Comparative summary of analytical results on apple varieties, 1920-1925*

Year	Mean summer tempera- ture (° F.)	Sunshine (percent- age of normal)	Composition of crop, ranked in order of amount of each constituent *		
			Total sugar	Acid	Relative astrin- gency
1920.....	64.0	101.7	5	4	( <sup>b</sup> )
1921.....	68.2	105.5	2	1	( <sup>b</sup> )
1922.....	65.9	97.9	4	3	2
1923.....	65.4	108.7	1	2	4
1924.....	62.9	102.1	6	5	1
1925.....	66.3	105.0	3	6	3

\* Rank of 1 indicates largest number of varieties having maximum content of constituent named, 2 next largest, and so on.

<sup>b</sup> Data on astringency not obtained in 1920 and 1921.

It is not to be inferred from what has just been said that every variety without exception behaved in the manner indicated. There were exceptions, but the general result was so clear cut as to compel the conclusion that the behavior of the group of varieties was a physiological response to the climatic environmental factors. Some 70

of the apples here discussed were included in the group of varieties employed. In a broad general way, they responded to the varying climatic conditions in the same manner as did the other members of the group. It would be expected on the basis of Shaw's theory that groups whose optimum temperatures are very close to the mean temperature would respond to greater amounts of sunshine by increased storage of the products of photosynthetic activity in the fruit. Such a result would scarcely be expected, on the basis of Shaw's theory, in groups having optimum temperatures far below those occurring at Washington. A detailed comparative examination of the analytical results for each of the temperature groups in the various years is necessary in order to determine whether the behavior of high and low temperature groups under a given set of conditions is similar or widely divergent.

Such a comparison presents difficulties, for the reason that all the varieties concerned were not analyzed in every year. Thirty of them were analyzed in 2 years only, 19 in 3 years, 27 in 4 years, 18 in 5 years, 4 in 6 years. Data upon astringency were not obtained in 1920 and 1921 except in a few cases. The results of a comparison consequently can not be as conclusive as they would be if the data for every variety were complete, but any pronounced tendencies will be apparent.

#### RELATION OF CHEMICAL COMPOSITION TO DESSERT QUALITY

Shaw has made in his second paper (11) practically the only attempt to be found in the literature to define the relation of "quality" in apples to their chemical composition. To summarize his statement, the apple of high dessert quality is low in insoluble and high in soluble solids, high in total sugars and acid, with a definite ratio between their amounts and with sucrose making up approximately one-third the total sugar content. Good keeping quality is associated with a high content of soluble solids and with a relatively high proportion of the sugar present in the form of sucrose. This statement probably presents as satisfactory a definition of quality in terms of chemical composition as it is possible to make and has been generally accepted as correct. It has been pointed out elsewhere (1) that the inclusion of data upon the tannin content of the fruit and the presentation of the figures for acidity, astringency, and sugar in the form of a ratio is a material aid in formulating a conception of the character of the fruit.<sup>3</sup>

It follows from Shaw's definition of quality that within a given variety the specimens having the higher content of soluble solids, total sugars, sucrose, and acid are of better dessert and storage quality than are those of a lower content of all these constituents, and that it is justifiable to rank a group of samples of a given variety as best, second best, and so on down to poorest on the basis of decreasing content of these constituents. This method of reasoning

<sup>3</sup> Chandler (3) is inclined to minimize the importance of the sugar-acid ratio as a factor in determining quality in apples, pointing out that the sugar-acid ratio in Green Newtown is approximately the same as in Ben Davis and that Rome Beauty, Fameuse, McIntosh, Jonathan, and Northern Spy have about the same ratio despite their differences in quality. His criticism appears to be based on a misunderstanding of the purpose and function of the sugar-acid ratio, which is an index of quality within the variety rather than between varieties. That Yellow Newtown and Ben Davis have like sugar-acid ratios tells nothing as to their comparative quality; but if several lots of either variety are compared among themselves, they will be found to rank in quality very nearly in accordance with the results of determinations of the sugar-acid ratio, going over to insipid or tart as the balance between the two rises or falls.



was followed by Shaw in comparing the analytical data on samples of the same variety from various localities. It would appear to be legitimate to apply the same process to the product of the same trees over successive years. This has been done in the present instance, and in the subsequent discussion a given variety is considered as having highest dessert quality in that year in which its total sugar, sucrose, and acid were highest, and as ranking progressively lower in quality with decrease in amount of these constituents.

#### CHARACTER OF THE 1923 CROP

Since 1923 was found to be the year of maximum total sugar, next to maximum acidity, and minimum relative astringency (ratio of total astringent substances to total sugar) in the study to which reference has been made, examination of the data of Table 1 may begin with a determination of the extent to which the same condition is found in the various temperature groups.

TABLE 3.—*Number of varieties in the various temperature groups having maximum and next to maximum total sugar; maximum and next to maximum sucrose; maximum and next to maximum acid content; minimum and next to minimum relative astringency in 1923*

Group (°F.)	Number analyzed in 1923 and in one or more other years	Number showing in 1923—							
		Maximum total sugar	Next to maximum total sugar *	Maximum sucrose	Next to maximum sucrose *	Maximum acid	Next to maximum acid *	Minimum relative astringency	Next to minimum relative astringency *
52.....	None.	0	0	0	0	0	0	0	0
53.....	5	4	0	4	0	4	1	2	1
54.....	4	4	0	3	0	2	0	3	1
55.....	8	5	2	5	2	5	1	3	2
56.....	9	7	0	5	3	6	0	4	2
57.....	11	6	3	8	2	3	2	5	2
58.....	8	4	2	6	0	3	1	2	1
59.....	7	6	0	4	1	4	0	6	1
60.....	8	8	0	5	2	4	1	6	1
61.....	3	2	1	3	0	1	1	0	0
62.....	6	4	2	3	2	2	2	3	2
63.....	1	0	0	0	0	0	0	0	0
64.....	4	2	2	4	0	1	1	2	0
65.....	2	2	0	2	0	1	1	2	0
66.....	1	0	0	1	0	1	0	0	0
67.....	1	1	0	0	1	0	1	1	0
Total.....	78	55	12	53	13	37	12	39	13

\* Only those varieties which were analyzed in four or five years are included in tabulating next to maximum and next to minimum values.

Table 3 brings together the results of an examination of the analyses made in 1923 in all the groups for the occurrence of maximum total sugars, sucrose, acidity, and minimum relative astringency. Of the 78 varieties analyzed in that year, 55 had maximum total sugar, 53 maximum sucrose, 37 maximum acidity, and 39 minimum relative astringency. In the case of each of these constituents there is also a number of varieties which had next to maximum amounts in 1923; in those varieties which were analyzed in four or five years 12 had next to maximum acidity, 13 had next to minimum astringency, 12 had next to maximum total sugars, and 13 had next to maximum

sucrose. Consideration of maxima alone shows clearly that there was a very pronounced tendency toward attainment of high total sugar and sucrose content in 1923. What is of immediate interest in the present connection is the fact, apparent from the tabulation, that high sugars and high acid content, with accompanying low astringency, are fully as frequent in the low-temperature groups as in those having optimum temperatures close to the mean for the year. In other words, the low-temperature groups utilize an exceptional increase in amount of seasonal sunshine, when grown at materially higher temperatures, just as do the groups for which the temperature is at or near the optimum.

#### CHARACTER OF THE 1921 CROP

In the study of the large group, 1921 ranked next after 1923 in the amount of total sugar and sucrose present in the crop as a whole, while the acidity was higher than in any other year of the period. Since 1921 was the hottest year of the period, with a mean temperature of 68.2° F., it would be expected, if the optimum-temperature theory holds, that the large excess of heat received by the groups having low optimum temperatures would unfavorably affect their total sugar, sucrose, and acid content. The data are summarized in Table 4.

TABLE 4.—*Number of varieties in the various temperature groups having maximum total sugar, sucrose, and acid content in 1921 (data on astringency not obtained)*

Group (°F.)	Number analyzed in 1921 and in 1 or more other years	Number showing in 1921—		
		Maximum total sugar	Maximum sucrose	Maximum acid
52.....	None	0	0	0
53.....	1	1	1	0
54.....	1	<sup>a</sup> 0	<sup>a</sup> 0	0
55.....	1	1	0	1
56.....	1	<sup>a</sup> 0	<sup>a</sup> 0	0
57.....	3	<sup>b</sup> 1	<sup>b</sup> 0	1
58.....	2	1	0	0
59.....	3	<sup>b</sup> 1	2	2
60.....	2	<sup>c</sup> 0	1	2
61.....	3	<sup>b</sup> 2	<sup>b</sup> 1	1
62.....	4	<sup>b</sup> 2	2	<sup>b</sup> 3
63.....	—	2	1	1
64.....	4	2	<sup>a</sup> 2	3
65.....	1	0	0	1
66.....	None	0	0	0
67.....	1	0	0	0
Total.....	29	<sup>d</sup> 13	<sup>e</sup> 10	<sup>b</sup> 15

<sup>a</sup> Exceeded in amount only by 1923.

<sup>b</sup> 1 other had a higher amount only in 1923.

<sup>c</sup> 3 others had higher amounts only in 1923.

<sup>d</sup> 8 others had maximum in 1923 with next to maximum in 1921.

<sup>e</sup> 7 others had maximum in 1923 and next to maximum in 1921.

The numbers in the various groups examined in 1921 are not large, only a single variety in some of the low and a few of the high-temperature groups. Of a total of 29 analyzed, 13 had their maximum sugar content while 8 others had next to maximum, having had their maxima in 1923. Ten had maximum sucrose, six others next to maximum, with maxima occurring in 1923. Fifteen had maximum acidity and one next to maximum. In all the groups there is a very apparent occurrence of maximum total sugars and sucrose in this

year with next to maximum amounts in those varieties which had their maxima in 1923. Considering the results for the analyses of 1923 and 1921 together, it is clear that nearly all the varieties analyzed in the two years had their maximum sugar content in one or the other of the two rather than indiscriminately over the other years. It is also clear that the high temperature of 1921 (68.2° F.) did not operate to prevent attainment of high total sugar and sucrose content on the part of many members of the low-temperature groups.

#### CHARACTER OF THE 1924 CROP

The year 1924 was the year of minimum mean summer temperature, 62.9° F., for the period covered by the climatic study. It was the year in which the crop of the varieties analyzed in that study, collectively considered, had minimum total sugar content, next to minimum sucrose and acid content, and maximum astringency. The high acidity which is characteristic of immature fruit was notably absent; instead, acidity was low in practically all varieties. The decline in mean temperature below the normal for the season should bring the groups having low to medium optimum temperatures into better adjustment with the locality and should have the opposite effect upon the high-temperature groups, if the optimum temperature theory holds. Table 5 indicates for the various temperature groups the extent to which their composition in 1924 followed that of the collective group. Of the 75 varieties analyzed in that year, 46 had minimum total sugar, 37 had minimum and 12 next to minimum sucrose, 26 minimum and 16 next to minimum acidity, and 45 maximum and 10 next to maximum astringency. Next to maximum and next to minimum values have been considered only in the case of varieties in which four years' analyses were made.

TABLE 5.—*Number of varieties in the various temperature groups having minimum total sugar, minimum or next to minimum sucrose, minimum or next to minimum acid content, and maximum or next to maximum relative astringency in 1924\**

Group (°F.)	Number analyzed in 1924 and in one or more other years	Number showing in 1924—			
		Minimum total sugar	Minimum or next to minimum sucrose	Minimum or next to minimum acid	Maximum or next to maximum relative astringency
52.....	1	1	0	1	1
53.....	5	5	3	2	3
54.....	5	4	4	3	5
55.....	7	4	4	4	5
56.....	9	5	4	4	4
57.....	8	4	5	6	6
58.....	6	2	4	2	5
59.....	5	2	4	1	4
60.....	9	8	8	5	7
61.....	4	1	5	2	3
62.....	6	5	2	4	4
63.....	None	0	0	0	0
64.....	6	3	4	4	5
65.....	2	1	1	2	2
66.....	1	1	1	1	1
67.....	1	0	0	1	0
Total.....	75	46	49	42	55

\* Next to minimum and next to maximum values in 1924 have been included only in cases in which data for four or five years were available for comparison.

There is consequently a tendency on the part of the whole group toward low sugar and acid content with high astringency, which is about as pronounced as the tendency toward the opposite condition in 1923. When the individual groups are examined, there are no pronounced differences between low-temperature and high-temperature groups. The greatest lack of correlation is in the middle of the series in the 58° and 59° groups, which show a tendency toward higher sugar and acid content than do the groups on either side. This is not sustained in the higher groups, the 63° and 64° groups showing no indication that their composition has been affected by a seasonal temperature near their optimum in a manner different from the other groups.

#### CHARACTER OF THE 1920 CROP

The year 1920 ranked next to 1924 in the number of varieties having low sugar content in the large group. In that study very nearly all the varieties analyzed in the two years had their minimum sugar and cane-sugar content for the whole period in one or other of the two years, but in greatest numbers in 1924. The summation in Table 6 shows that the number of absolute minima for sugars and acids were much less than in 1924. There are a number of next to minimum values in 1920 in the groups analyzed in four or more years, with minimum values occurring in 1924, and minimum and next to minimum values taken together predominate over higher amounts, except in the case of acidity. There is no marked difference between the results in the high-temperature and the low-temperature groups in these respects.

TABLE 6.—*Number of varieties in the various temperature groups having minimum total sugar, sucrose, and acid content in 1920 (data on astringency not obtained)*

Group (°F.)	Number analyzed in 1920 and in one or more other years	Number showing in 1920—		
		Minimum total sugar	Minimum sucrose	Minimum acid
52.....	1	0	1	0
53.....	2	1	1	1
54.....	4	0	2	1
55.....	2	0	1	0
56.....	6	3	3	1
57.....	6	1	3	0
58.....	4	2	2	1
59.....	2	1	2	1
60.....	2	0	1	1
61.....	2	2	2	0
62.....	None.	0	0	0
63.....	1	0	0	0
64.....	2	1	1	0
65.....	1	0	0	0
66.....	None.	0	0	0
67.....	None.	0	0	0
Total.....	35	11	19	6

Taken collectively, 1924 and 1920 have nearly all the minimum values found at any time for total sugars, sucrose, and acidity for the varieties analyzed in these years regardless of the temperature groups to which they are assigned. Further, a very large majority of the varieties analyzed in 1923 and 1921 have the maximum amounts

of these constituents found at any time in one or the other of these years. This is merely another way of saying that all the varieties examined respond to the climatic conditions of the successive years in the same general way. The year having most favorable conditions for photosynthetic activity is the year having the largest number of maximum values for sugars and a considerable number of next to maximum values. The year which was next in rank with respect to conditions for photosynthesis was second in number of maximum sugar values, with a very considerable number of next to maximum values. The two years which were least favorable for photosynthetic activity are those characterized by the occurrence therein of minimum and next to minimum values for sugars and acid in nearly all the varieties analyzed in these years. Members of the low-temperature groups behave exactly as do those of the high-temperature groups in this respect. The exceptions to this general situation are so few that they can be named: Arkansas Black of the 63° group has minimum sugar in 1923; King David of the 59° group and Gano of the 64° group in 1921; Scott Winter of the 54° group and Sutton of the 56° group have maximum sugar in 1924, while Dudley of the 53° group and Golden Sweet and Swaar of the 58° group have maximum sugar in 1920. These are so few in number and so scattered throughout the whole extent of the groups as to be entirely without significance.

#### CHARACTER OF THE 1922 CROP

In the study of effect of climatic conditions on composition, the years 1922 and 1925 were found to be characterized by absence of either maximum or minimum contents of sugar, acid, and tannin. This is the general situation in 1922. Of the 78 varieties analyzed in that year, 60 were analyzed in at least two other years. Examining the data for these, it is found that only two of the 60 had maximum sugar, Gideon of the 54° and Haas of the 59° group. A number had their minima, McMahon of the 55° group, McIntosh and Wealthy of the 56° group; Babbitt, Boiken, and Jefferis, 57°; Red June and Swaar, 58°; Delicious, 59°; Fallawater, 60°; Ortle, 61°; Ingram, 62°; Oliver Red, 64°; Collins, 65°; and Terry, 67°. The distribution of these 15 varieties through all the groups makes it clear that the conditions of the year had no specific depressing effect upon sugar formation and storage in any particular temperature group.

#### CHARACTER OF THE 1925 CROP

Of the 44 varieties analyzed in 1925, 43 had been analyzed in at least two other years. Thirty of the number show values for sugars intermediate between the high figures found in 1923 and 1921 and the low figures found in 1924 and 1920. The remaining 13 are divided between the two extremes. Six had the highest sugar content found in any year in which they were analyzed. These are McMahon of the 55° group; Northern Spy, 56°; Monmouth and Roxbury Russet, 57°; Mother, 58°; and Paragon, 64°. In this year, seven had the minimum sugar content found at any time. These were Walbridge of the 54° group; Scott Winter, 55°; McIntosh, 56°; Hubbardston and Williams, 57°; Kinnard, 59°; and Huntsman, 62°. Maximum and minimum values occur side by side throughout the

series of temperature groups in such a fashion as to forbid the conclusion that they are other than responses to uncontrolled nonclimatic factors affecting specific individuals and not a response to any generally operative factor such as the temperature of the season.

#### GENERAL DISCUSSION

It is clear from the foregoing analysis of the results that the various groups of apples behaved in very large measure as a unit with respect to the composition of the crop throughout the period of observation. Two years having mean temperatures differing considerably—1923 with  $65.4^{\circ}$  and 1921 with  $68.2^{\circ}$ —are years characterized by high total sugar, sucrose, and acid content in a very large majority of varieties irrespective of the temperature groups to which they have been assigned. Varieties with a maximum in one of these years quite generally show next to maximum figures in the other. On the other hand, the two years having the minimum temperatures occurring during the period—1920 with  $64^{\circ}$  and 1924 with  $62.9^{\circ}$ —are characterized by low content of sugars and acid in a large majority of the varieties analyzed. Most of these, high-temperature and low-temperature varieties alike, show a minimum in one of the years and next to minimum in the other. Exceptions to these general statements occur in some numbers, but the departures from the general mass level occur in both directions and in all temperature groups, thus definitely disposing of any possibility that they can be due to the operation of a single factor.

In all that has been said thus far the comparison of the crops of the several years has been based upon the analytical data on the assumption that the amounts of total sugars, sucrose, acid, and tannin present in the expressed juice of a variety in any year is an index of the quality of the crop, as compared with similar analyses of the same variety in other years. It has already been pointed out that this is a legitimate assumption and that to deny it amounts to denying that chemical composition and quality have any discoverable relation. But there is considerable collateral evidence as to the comparative dessert quality and storage behavior of the crops of the years under discussion.

Representative tree-run samples of the crop, not only of the varieties here employed but also of all the apples in the variety collection at Arlington, have been examined by H. P. Gould, of the Office of Horticulture, each year, beginning with 1922. It has been Gould's custom to make somewhat full notes upon the individual varieties as they are inspected each year. These notes deal with size, color, texture of flesh, and dessert quality, primarily in comparison with his conception of the variety as such conception has taken form in the course of 25 years of continuous observation and study which has thoroughly familiarized him with nearly all these varieties throughout their range. Gould has kindly permitted an examination of these records and has discussed with the writer his estimates of the character of the crops of the various years. In a very considerable number of cases his notes upon the 1923 crop comment upon the exceptionally fine color, finish, and dessert quality of the sample of a variety. Those upon the 1924 crop as frequently record the statement that the sample was deficient in

color, size, flavor, dessert quality, and in nearly all the attributes of typical well-grown specimens of the variety. In both years these comments are made upon varieties distributed throughout all the temperature groups. Gould summarizes his estimates of the crops of the two years by the statement that as a whole the fruit of the 1923 crop of the Arlington orchard was of materially higher quality than that of any other year covered by his notes, while that of 1924 was as a whole and with few exceptions markedly inferior in appearance, color, and quality. J. R. Magness, of the same office, who has employed a considerable number of the more important commercial varieties in storage experiments during the years in question, states that the 1923 fruit was normal in its storage behavior, while that of 1924 was decidedly inferior in keeping quality, breaking down prematurely and failing entirely to develop the normal dessert quality of the variety. The independent judgments of these two gentlemen, therefore, support and confirm estimates of the character of the fruit based upon the results of comparison of the analytical data.

It appears from the data here presented that mean summer temperature is not the sole or dominant factor in determining the performance of trees during a season, as measured by the composition of the fruit. It is not possible to find any definite or consistent relationship between the amount of heat received in any season and the chemical composition of the fruit which indicates that any group of varieties has a fixed response to a definite seasonal temperature. On the contrary, the chemical results throughout the groups are in the great majority of cases directly opposite to expectation based on the assumption that temperatures control chemical composition. The normal mean summer temperature at Washington practically coincides with the highest temperatures of Shaw's groups. In consequence, most of the groups are growing under conditions in which they receive 2 to 10 degrees of heat daily in excess of their supposed optima. In accordance with theory, an excess of 2 degrees over the optimum temperature results in very evident alterations in size, form, coloration, and storage behavior as well as in chemical composition and quality. If this be true, varieties of the low-temperature groups, those with optima of 52° to 60° F., for example, should show consistent, rather wide departures from their normal character and composition year after year. In years of excessively high temperatures, as 1921, they should depart still further from normal, and such departures should become evident at higher levels in the ascending scale of temperature groups. In years of sub-normal temperatures, as 1920 and 1924, the opposite result should be observed. Groups with optimum temperatures near that actually occurring in the season should be enabled to attain their optimum development; low-temperature groups should show smaller departures from the normal, while the high-temperature groups should be adversely affected by the lower temperatures.

But the actual results entirely fail to bear out these assumptions based on the optimum-temperature theory. Low-temperature varieties develop maximum sugar in the hotter years and minimum sugar in the cooler years, just as do the high-temperature varieties. It would be entirely impossible, from examination of the analyses of any year, to assign the varieties concerned to their respective tem-

perature groups, or even to say that any given variety belonged to a group having an optimum temperature above or below that of the year in question. It would also be impossible to examine the analyses of the members of any temperature group over a series of years and reach therefrom any correct conclusions as to the temperature conditions, considered separately and apart from the general climatic complex, in the various years. On the contrary, one would be led by the theory to consider the temperatures of the various years as opposite to what they actually were.

These facts would appear to make necessary some modifications of the theory of optimum temperatures. Chandler (3, p. 589) has said, in commenting upon the classing of varieties into temperature groups:

While the fine discriminations of the table may not be justified, we can be reasonably certain that the varieties toward the top of the table are adapted to a low mean summer temperature, and those toward the bottom to a high mean summer temperature.

This statement, in which low-temperature and high-temperature groups are referred to as standing, respectively, toward the top and bottom of the table, can not be seriously questioned by anyone. But the individual variety, in the light of the results here reported, appears to be highly plastic in so far as adaptation to considerable variations in amount of seasonal heat received is concerned. Departures from the supposed optimum are not followed by definite and constant departures from normal composition. Heat-loving and cold-loving varieties behave as do those native to the latitude of the experiment; the chemical composition of their fruit in any season is a measure of the opportunity for photosynthetic activity afforded by the season, and there is no evidence that one differs from another in ability to utilize such opportunity. The results presented in the present paper and in accompanying papers show very clearly that throughout large groups of varieties there is a very considerable range of variation from year to year in the composition of the fruit of the same trees, and that these variations take the form of mass movements, large groups of individuals behaving as one. It has been shown that the direction and character of these mass variations are definitely related to the climatic conditions of the season, but the determining factor in the climatic complex is not temperature alone. Temperature enters into the results as a factor of secondary importance to sunshine, since the amount of sunshine is a direct measure of the opportunity for photosynthetic activity afforded by the season. In the latitude of Washington, these variations in chemical composition of the crop are striking both as regards their absolute and their relative amounts, and their range is as wide in varieties supposedly perfectly adapted to the region, such as York Imperial and Lawyer, as it is in varieties from the extreme northern and southern limits of commercial apple growing, such as Arctic or Dudley and Buckingham or Terry. Further, the variation in total sugars, sucrose, and acid occurring in the fruit of the same pair of trees at Arlington in the course of five or six years, in the case of the varieties studied by Shaw, is as great as he found it to be in fruit collected in one or two years over the entire commercial range of the variety. The chemical differences found by Shaw between York Imperial grown in Kansas, Tennessee, and Massa-



chusetts are not so wide as those between the crops of the Arlington trees in 1923 and 1924. His samples of Baldwin from Amherst, Mass., showed nearly as large a range in sugar content as did those from Ohio, Kansas, New Hampshire, and British Columbia, and all of them fell markedly below the 1923 and 1921 crops at Arlington despite the fact that the Arlington temperatures in these years were 9.5 and 12.2 degrees, respectively, above the supposed optimum for Baldwin. The Arlington samples ranged from 10.11 per cent total sugar in 1920 to 14.96 per cent in 1923, the Amherst samples in 1910 from 7.90 to 10.64 per cent, and those from five other States and British Columbia from 7.86 to 11.79 per cent. Hence all the differences in soil, culture, and climatic conditions represented by samples collected over a wide area are productive of smaller differences in sugar content than are found in the fruit of a single pair of trees when analyses are continued through a series of years of rather widely varying seasonal conditions.

There is nothing in the vegetative behavior or in the fruitfulness of any of the groups of varieties here studied that can be considered clearly indicative of any marked lack of adjustment to the conditions of the locality. With respect to the composition of the fruit, the variations which occur from year to year are definitely related to the climatic conditions of the various seasons, and all the varieties studied behave as a unit in the direction, extent, and character of the chemical modification produced by a given set of seasonal conditions. All the varieties, high-temperature and low-temperature groups alike, produce their best crops under the seasonal conditions which afford greatest opportunity for photosynthetic activity, their poorest crops under the least favorable conditions for such activity. If excess temperature were acting as a limiting factor in the case of a part of the varieties employed, the degree of uniformity of physiological response shown by the analyses could not be expected to occur. That it does consistently occur would seem to make it clear that the exact adjustment of varieties to definite quantities of summer heat postulated by the optimum-temperature theory does not exist. Instead, most apple varieties have such a capacity of adaptation to varied climatic conditions that they can attain normal development under quite varied summer temperature conditions, subject only to the limitations upon such development resulting from the variations in seasonal conditions which occur from year to year.

#### SUMMARY

The optimum-temperature theory as applied to the development of apples asserts that mean summer temperature is the dominant factor of climate which determines quality in apples. It assumes that apple varieties have such exact adaptations to climatic conditions that any variety can attain its optimum development only at a certain definite mean summer temperature, exhibiting definite physical and chemical departures from normality with consequent lowering of quality when grown at temperatures above or below this optimum.

Ninety-eight varieties of apples belonging to 16 groups having supposed temperature optima from 52° to 67° F., inclusive, grown together under uniform controlled conditions at the Arlington

Experiment Farm, Rosslyn, Va., near Washington, D. C., under an average mean summer temperature of 64.5° have been studied over a period of six years. The accumulation of analytical data upon the composition of the fruit has been accompanied by a study of the climatic conditions of the years covered by the work.

As the mean summer temperatures at the Arlington farm very closely approximate the optima for the high-temperature groups but very greatly exceed those for the low-temperature groups, the optimum-temperature theory would assume that there would be a very decided contrast in the results observed with the two groups. Varieties of the low-temperature groups should display marked inability to attain normal chemical composition and dessert quality, while the varieties of high-temperature groups should attain a development very close to the optimum for the respective varieties. No such contrast appears in the results.

Mean summer temperature as a separate factor has little influence in determining the composition of the crop. The chemical character of the crops of the low-temperature groups in the warmest year (1921) and the coldest year (1924) are exactly the opposite of expectation based on the assumption that temperature is the dominant factor in determining composition. Years of closely identical mean summer temperatures show very considerable differences in the chemical character of the crop.

The composition of the fruit of all varieties shows considerable variations from year to year. The direction and extent of these variations are determined by the opportunities for photosynthetic activity afforded by the conditions of the season, and in responding to the seasonal conditions all the varieties, high-temperature and low-temperature groups alike, behave as a unit. Climatic conditions favoring the attainment of high quality as measured by total sugar, sucrose, acid, and astringency content in one group favor the attainment of a like condition in all groups regardless of their supposed temperature adaptations. Conditions which depress quality as measured by these constituents in one group exert a like depressing effect upon all groups.

The series of years covered by the experiment present an exceptionally wide range in the extremes of seasonal conditions which have occurred in them. Throughout the whole period, all the varieties included in the study have shown a high degree of uniformity in their behavior. In rate of growth, vigor, and health of tree, fruitfulness, and composition of the fruit there have been no indications that any of them were out of adjustment with the environment.

Varieties of the apple possess much greater capacity of adaptation to varied summer temperature conditions than is assumed by the optimum-temperature theory.

#### LITERATURE CITED

- (1) CALDWELL, J. S.  
1928. CHEMICAL COMPOSITION OF APPLE JUICES AS AFFECTED BY CLIMATIC CONDITIONS. *Jour. Agr. Research* 36:289-365.
- (2) ———  
1928. THE CHEMICAL COMPOSITION OF AMERICAN-GROWN FRENCH CIDER APPLES AND OTHER APPLES OF LIKE CHARACTER. *Jour. Agr. Research* 36:391-406.

- (3) CHANDLER, W. H.  
[1925.] FRUIT GROWING. 777 p., illus. Boston, New York [etc.].
- (4) FRASER, S.  
1924. AMERICAN FRUITS, THEIR PROPAGATION, CULTIVATION, HARVEST-  
ING AND DISTRIBUTION. 888 p., illus. New York.
- (5) GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D.  
1922. THE FUNDAMENTALS OF FRUIT PRODUCTION. 686 p., illus. New  
York.
- (6) LEHENBAUER, P. A.  
1914. GROWTH OF MAIZE SEEDLINGS IN RELATION TO TEMPERATURE.  
Physiol. Researches 1: 247-288.
- (7) LIVINGSTON, B. E.  
1916. PHYSIOLOGICAL TEMPERATURE INDICES FOR THE STUDY OF PLANT  
GROWTH IN RELATION TO CLIMATIC CONDITIONS. Physiol. Re-  
searches 1: 399-420.
- (8) ——— and SHREVE, F.  
1921. THE DISTRIBUTION OF VEGETATION IN THE UNITED STATES, AS  
RELATED TO CLIMATIC CONDITIONS. 590 p., illus. Washington,  
D. C. (Carnegie Inst. Wash. Pub. 284.)
- (9) SEARS, F. C.  
1917. PRODUCTIVE ORCHARDING. Ed. 2, 315 p., illus. Philadelphia and  
London.
- (10) SHAW, J. K.  
1910. VARIATION IN APPLES. Mass. Agr. Expt. Sta. Ann. Rpt. 22: 194-  
213, illus.
- (11) ———  
1911. CLIMATIC ADAPTATIONS OF APPLE VARIETIES. Mass. Agr. Expt.  
Sta. Ann. Rpt. 23: 177-245, illus.





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## CHEMICAL COMPOSITION OF AMERICAN-GROWN FRENCH CIDER APPLES AND OTHER APPLES OF LIKE CHARACTER<sup>1</sup>

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### INTRODUCTION

One of the investigations in progress in the fruit and vegetable utilization laboratory of the Office of Horticulture has had as its object the devising of better methods for making unfermented apple and grape juices for use as beverages. Very early in the work it was realized that one of the serious difficulties to be overcome in making acceptable beverage apple juices arises from the deficiency in astringent substances characteristic of nearly all dessert and of most of the culinary apples. In order to meet with popular approval, a beverage juice must possess a rather definite relationship or balance between the amounts of sugar, acid, and astringent substances it contains, since judgments of taste or palatability of a liquid are based upon the perception by the nerves of taste of its sweetness, acidity, and astringency. Very few of the American dessert apples and comparatively few of those grown for culinary uses have sufficient quantities of astringent substances, when the condition of cider ripeness has been attained, to make their juices acceptable. As a whole their juices are insipid or acid-sweet, not because of excessive sugar content, but because of deficiency of tannin and in many cases of acid also. In order to make acceptable beverages, such juices must be given the requisite balance between constituents by blending with other juices having larger astringent content. It is relatively easy to find fruit of sufficiently high acidity for use in correcting any deficiency in that respect, but fruits capable of supplying added astringency must be sought outside the range of the varieties generally cultivated in the United States.

The French cider makers have long recognized the necessity for the blending of juices in order to make balanced and palatable fermented ciders, and the French pomological literature classifies the varieties employed for cider making into groups based on their outstanding characters, as "douce," "acide," "amère," "douce-amère," and the like. Among American workers, Alwood (1, 2)<sup>2</sup> and Price and Ellett (7) have recognized that apples of such special types are a prerequisite to the making of fermented apple beverages of a type and quality comparable with the French products. That such material is equally

<sup>1</sup> Received for publication July 28, 1926; issued May, 1928. This paper is the fifth in a series of studies on fruit juices.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 406.

indispensable to the making of unfermented beverage juices of the highest quality does not seem to have been recognized.

In the course of the analytical work upon apples carried on during the last seven years, an attempt has been made to obtain as much information as possible in regard to apples of high astringent content, and a considerable number of apples of this character have been analyzed at various times as material and opportunity offered. The analyses include a number of French cider apples ("French crabs"), for some of which it is believed no analyses of material grown in America can be found in the literature, together with a number of crab apples and highly astringent apples of various origins.

#### MATERIAL AND METHODS

The fruit employed in the analyses was grown in the apple variety collection of the Office of Horticulture, Bureau of Plant Industry, at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. The varietal identifications have been verified by tracing the scions to their sources in all doubtful cases and by repeated checking of the fruit with varietal descriptions. The French cider apples are fairly well represented in the Arlington orchard, many of the trees having been grown directly or indirectly from scions brought from France by Alwood (1).

In order to obtain some indication of the degree of modification in character of these apples occurring when grown in widely differing environments, samples of fruit were obtained in 1923 from the collection growing at Blacksburg, Va., on the grounds of the Virginia Agricultural Experiment Station, from Alwood's original stocks, for comparison with fruit grown at the Arlington farm. Through the kindness of H. L. Price and other members of the staff of the Virginia station, the entire crop of the varieties in fruit in 1923 was harvested and shipped to Washington. The trees had been in sod for some years, the grass being cut for hay or pastured. They were top-worked upon standard varieties in 1900, the grafts consequently being 23 years old at the time of sampling. Descriptions of trees and fruits and analyses of the whole fruits as grown at Blacksburg were published in 1909 by Price and Ellett (?) at the time the trees were 8 years old. Their analyses, with a single analysis of Moulin-à-Vent by Alwood, Davidson, and Moncure (2), made upon the first crop borne by the Blacksburg trees, would appear to be the only analyses of American-grown French apples in the literature.

The general method of handling the material employed in the analyses of the fruit grown at the Arlington farm and the cultural treatment of that orchard have been fully described elsewhere (4). The fruit was picked at the stage of picking ripeness and held in a cool basement room until it had reached proper condition for pressing. The samples were tree run, the quantities ranging from 2 to 10 bushels, and the only sorting done was confined to the discarding of partially decayed fruits when such were present. In the case of the material of Bramtot, Launette, and Rouge du Landel, grown at Blacksburg, the fruit was ready for pressing when picked. Shipment by freight to Washington required five or six days, during which the temperature in the car ranged rather high, and the fruit consequently showed considerable decay and was slightly past prime

condition for pressing when received at Washington. It was therefore pressed immediately. With these exceptions, the fruit was all in prime cider-ripe condition when the pressing was done. Care was employed to obtain uniform pressing of the samples, and the analyses were begun immediately. The bulk of the juice obtained was preserved by pasteurization, and a repetition of the analyses after pasteurization has incidentally served as verification of the determinations of total sugar and titratable acidity made upon the fresh juices.

It was necessary to determine for the present purpose what is meant by an astringent apple. In the analyses made in this laboratory, the extremes of total astringency encountered have been 7.4 and 955 mgm. per 100 c. c. It is necessary to draw a line somewhere across this range and to consider everything above it as in the astringent class. This must be done arbitrarily. As culinary varieties rarely equal or exceed 125 mgm. of total astringents per 100 c. c. in juice when expressed at the cider-ripe stage, it is probably fair to fix the arbitrary limit at that figure. The present paper consequently includes not only analyses of French, Siberian, and other crabs, but also of all apples of whatever origin which have been found to have a total astringency consistently equaling or exceeding 125 mgm. per 100 c. c. For the sake of completeness a number of analyses previously published in communications from this laboratory have been included in the tables.

It should be clearly understood that the fact two juices have identical amounts of total astringent materials does not necessarily imply that they are equally astringent to taste, even though their acid and sugar content should also be identical. Total astringency is made up of the two components, tannins and astringent nontannins. Nothing is known as to the relative effects of equal amounts of true tannin and of astringent nontannin upon the nerves of taste, and it is possible, indeed certain, that quite different effects upon the nerves of taste are produced by two juices having the same total astringency but differing in the amounts of tannin and nontannin. Moreover, the term "astringent nontannins" is a collective one, applied to all substances which reduce  $\text{KMnO}_4$  but are not precipitated by gelatin. The nature of these substances is largely unknown, but it is certain that they are of varied character and that they differ markedly in their effect upon the nerves of taste. The bitter-sweet group of apples, for example, contain varying amounts of a bitter constituent, probably glucosidal in nature, which is not encountered in apples of other types. The nature as well as the relative amounts of the astringent substances consequently vary from variety to variety, and as a result a determination of the total amount does not give an accurate measure of the astringency of the juice as measured by the sense of taste.

Determinations of total astringency are therefore like determinations of total sugars in which varying and unknown amounts of sucrose, dextrose, levulose, and pentoses are present. Such determinations tell nothing as to the relative sweetness of the mixtures as determined by the sense of taste. In the case of sugars a very accurate idea of the sweetness of a mixture is attainable by determining the amounts of the individual components, but so little is known of the astringent substances of fruits that a similar treatment can not as yet be applied to them.

## SEASONAL CONDITIONS IN RELATION TO ANALYTICAL RESULTS

The analyses presented herewith were made in the years 1920 to 1925, inclusive. In a considerable number of cases several analyses were made upon a given variety, either in consecutive years or at intervals throughout the period occupied by the work; in other cases only a single analysis was made. In other investigations which were proceeding concurrently with the present work (4, 5), a detailed study of the climatological conditions of the years 1920 to 1925, inclusive, was made with respect to the effects of the seasonal conditions upon the composition of the fruit of somewhat more than 200 varieties of apples. As a result of this investigation it was found that the seasonal conditions of the growing period determine the composition of the crop of the year, all the varieties employed behaving in very high degree as a unit. In consequence, the six years covered by the study can be ranked in order with respect to the sugar content of the fruit produced. Acid and astringency content of the fruit is correlated with sugar content in a very definite manner, the correlations being consistently maintained throughout the period of the work.

From the results of these investigations it appears that of the six years, 1923 was that characterized in a large majority of varieties by the maximum sugar content, maximum sucrose content, next to maximum acid content, and minimum astringency content found in the fruit during the six-year period. The year 1921 stood next to 1923 in point of sugar content of the crop, 1925 third, 1922 fourth, 1920 fifth, and 1924 sixth and lowest. With the minimum sugar content found in 1924 there was associated minimum or next to minimum sucrose and acidity content and maximum astringency. As these results with respect to composition of the fruit of a large group of varieties have been shown to be definitely related to the seasonal conditions of the various years, and as much of the fruit employed in the present work was grown in the Arlington orchard, subject to the same conditions, it may be assumed that these analyses reflect seasonal conditions in the same manner and to the same degree as do those presented in the climatological study. Hence analyses made in 1923 or 1921 represent the composition of the crop as grown under exceptionally favorable seasonal conditions for accumulation of high sugar content and attainment of high quality for the variety, those made in 1924 or 1920 represent the opposite extreme of highly unfavorable conditions for storage of sugar, and those made in 1922 or 1925 an intermediate condition. An isolated analysis in any particular year should be evaluated in the light of these statements, which are based upon evidence presented in detail in the papers to which reference has previously been made (4, 5).

## FRENCH CIDER APPLES

Analytical data upon 21 varieties of French cider apples are presented in Table 1. In the case of 5 varieties material grown at both Blacksburg and the Arlington farm was used; in the others fruit from only one location was employed. The material grown at Blacksburg was analyzed in 1923 only, while some of the varieties grown at the Arlington farm were analyzed in two, three, or four



TABLE 1.—Analyses of apples of French cider varieties grown at the Arlington Experiment Farm, Rosslyn, Va., and at Blacksburg, Va.

Variety (apples were grown at Arlington farm except as indicated in footnotes)	Date picked	Date analyzed	Constituents (per cent)						Total solids	Non-tannin astrin-gency	Acid-astrin-gency-sugar ratio
			Reducing sugar	Sucrose as invert sugar	Total sugar	Acid	Total astrin-gency	Tannin			
Amère du Surville.....	Sept. 23, 1922	Dec. 14	8.98	0.94	9.92	0.410	0.3584	0.2136	0.1248	13.91	1:0.82:24.2
	Sept. 4, 1923	Sept. 19	9.70	1.64	11.34	.248	.7400	.4220	.3180	13.21	1:2.98:45.8
	Sept. 1, 1923 <sup>a</sup>	Sept. 11	9.58	1.01	10.59	.231	.8050	.5890	.2170		1:3.47:45.8
Bedan des Parts <sup>a</sup> .....	Oct. 18, 1925	Nov. 8	8.97	2.01	10.98	.148	.4390	.2705	.1565		1:2.04:74.2
	Oct. 10, 1925	Oct. 17	11.06	1.82	12.88	.176	.4740	.2140	.2600		1:2.09:73.2
	Sept. 7, 1923	Sept. 21	10.10	1.54	11.64	.215	.3132	.1099	.1433	16.87	1:1.378:54.1
Bonne de Frenilles.....	Aug. 28, 1923	Sept. 9	9.14	1.37	10.51	.146	.4070	.2745	.1325	11.40	1:2.75:54.1
	Sept. 11, 1923	Sept. 24	7.73	3.61	11.34	.330	.3280	.2050	.1230	13.83	1:6.99:34.3
	Sept. 6, 1923	Sept. 19	11.84	3.69	15.63	.316	.3885	.1735	.1650		1:1.07:49.4
Bramtot.....	Sept. 2, 1923 <sup>a</sup>	Sept. 10	11.94	4.10	15.94	.187	.4600	.2432	.2168	17.82	1:2.46:85.2
	Sept. 16, 1923	Sept. 29	11.32	.69	11.01	.343	.6100	.4010	.2090	12.93	1:1.77:32.1
	Oct. 4, 1923	Oct. 18	11.53	1.83	13.36	.318	.2780	.2691	.1157	14.68	1:1.97:63.6
D'Avrolles <sup>a</sup> .....	Aug. 27, 1923	Sept. 10	7.49	2.89	10.38	.370	.1568	.0568	.1000	15.58	1:0.87:32.3
	Sept. 16, 1923	Sept. 29	9.71	4.05	13.76	.317	.1474	.0677	.0797	14.47	1:0.42:37.2
	Sept. 28, 1922	Dec. 8	9.70	1.34	11.04	.817	.1045	.0893	.0852	15.18	1:0.18:13.5
Fréquin Lajoie <sup>a</sup> .....	Sept. 8, 1923	Oct. 9	9.13	2.71	11.84	.890	.1045	.0798	.0557		1:0.17:13.3
	Sept. 23, 1924	Oct. 4	8.53	2.13	10.66	.799	.0312	.0401	.0511	12.94	1:0.21:23.4
	Sept. 25, 1925	Oct. 29	8.70	1.54	10.24	.437	.0612	.0401	.0511	14.10	1:1.96:62.5
Hilaire.....	Sept. 2, 1923	Sept. 7	9.70	1.44	11.14	.178	.3480	.2215	.2800	16.01	1:1.74:37
	Sept. 3, 1923	Sept. 19	9.52	3.38	12.90	.350	.6100	.3200	.3140		1:2.55:63.3
	Sept. 7, 1924	Sept. 18	8.35	3.37	11.72	.185	.4720	.1850	.2400	15.09	1:1.70:57.9
Julien de Paulmier.....	Oct. 5, 1925	Oct. 12	8.80	3.52	12.32	.182	.3025	.0860	.2365		1:1.23:44.4
	Sept. 1, 1923 <sup>a</sup>	Sept. 7	8.35	3.05	11.63	.229	.3025	.0860	.2365	11.93	1:2.39:50.7
	Sept. 15, 1924	Sept. 24	14.43	1.21	16.04	.229	.6665	.3685	.3780	17.88	1:0.87:52.4
Launette.....	Sept. 11, 1923	Sept. 18	11.98	3.18	15.16	.163	.4050	.2800	.1720	15.77	1:2.48:6
	Sept. 1, 1923	Sept. 7	7.46	3.72	9.17	.163	.1820	.0850	.1141		1:0.87:52.4
	Sept. 1, 1923	Sept. 10	12.77	3.16	11.00	.250	.6400	.3200	.3110	21.13	1:2.5:61
Moulin-à-Vent.....	Sept. 7, 1923	Sept. 10	12.77	3.16	11.00	.250	.6400	.3200	.3110	21.13	1:2.5:61
	Sept. 11, 1923	Sept. 18	8.50	1.87	10.37	.164	.2980	.1000	.1620	11.20	1:0.3:9.58
	Sept. 15, 1924	Sept. 28	7.86	1.61	9.47	.988	.2984	.1339	.1645	14.36	1:2.03:57
Passé Reine.....	Sept. 16, 1924	Sept. 24	8.20	3.32	11.52	.202	.4210	.2628	.1482		1:1.16:43.5
	Sept. 21, 1920	Aug. 12	7.80	1.54	9.34	.348	.0716	.0716	.1255	9.51	1:0.47:23.3
	July 13, 1922	July 16	6.24	1.84	8.13	.358	.1635	.0819	.0819		1:1.09:32.5
Précoce de Tunis.....	July 16, 1923	July 26	7.76	3.87	11.63	.189	.3910	.1900	.2010	13.92	1:2.34:60
	Sept. 17, 1923	Sept. 22	8.15	3.18	11.33	.329	.5945	.3965	.1585	14.05	1:1.8:36
	Sept. 1, 1923 <sup>a</sup>	Sept. 7	8.80	3.10	11.90	.332	.1982	.0956	.1026	15.37	1:0.6:36.8
Reine des Pommes.....	Sept. 16, 1922	Oct. 21	10.23	1.89	12.23	.332	.2820	.1458	.1468	11.76	1:0.823:28.2
	Sept. 1, 1923 <sup>a</sup>	Sept. 8	8.20	1.60	10.00	.355	.2512	.1002	.1002	11.93	1:0.848:32.7
	Sept. 7, 1923	Sept. 15	8.23	1.45	9.68	.296	.2512	.1002	.1002		1:1.7:61.3
Rossignol <sup>a</sup> .....	Sept. 7, 1923	Sept. 10	7.58	2.91	10.49	.171	.2920	.1575	.1345		
	Sept. 7, 1924	Sept. 20									
	Sept. 7, 1924	Sept. 20									
Rouge du Landel.....	Sept. 7, 1924	Sept. 20									
	Sept. 7, 1924	Sept. 20									
	Sept. 7, 1924	Sept. 20									

<sup>a</sup> Analysis at Blacksburg, Va., quoted from Alwood, Davidson, and Moncreau (?).<sup>a</sup> Material from Blacksburg, Va.

years. Reports of analyses of the whole fruit of some of these varieties have been made by Price and Ellett (7). Analyses of material grown in France have been compiled by Power (6), by Truelle (8), and by Warcollier (9). For purposes of comparison, some of the results given by these authors are reproduced in Table 2. For the sake of completeness, data have been included for a few varieties analyzed by the French workers and by Price and Ellett which were not available for the present work.

TABLE 2.—*Summary of analyses of juices and fruit of French-grown French cider apples and of French cider apples grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va.*

Variety and product <sup>a</sup>	Total sugar	Acidity as malic <sup>b</sup>	Total astringency	Solids
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Amère du Surville:				
Juice (French).....	14. 19-15. 21	0. 158-0. 250	0. 300-0. 807	-----
Juice (Arlington).....	9. 92-12. 88	. 148-. 210	. 338-. 740	13. 91-15. 87
Juice (Blacksburg).....	10. 59	. 231	. 805	13. 21
Whole fruit (French).....	12. 20	. 350	. 0760	20. 2
Whole fruit (Blacksburg).....	9. 48-12. 40	. 464-. 778	. 570-. 760	19. 92-23. 65
Bedan des Parts:				
Juice (French).....	14. 02-16. 32	. 191-. 240	. 152-. 310	-----
Juice (Blacksburg).....	10. 51	. 146	. 407	11. 40
Whole fruit (French).....	12. 61	. 360	. 170	21. 60
Whole fruit (Blacksburg).....	11. 68	. 601	. 360	21. 58
Binet Blanc:				
Juice (French).....	17. 14	. 220	. 190	-----
Whole fruit (French).....	16. 48	. 380	. 150	19. 70
Whole fruit (Blacksburg).....	12. 38	. 396	. 130	19. 78
Binet Rouge:				
Juice (French).....	15. 30-17. 60	. 125-. 24	. 191-. 270	-----
Whole fruit (French).....	11. 93	. 25	. 160	20. 5
Whole fruit (Blacksburg).....	14. 12	. 546	. 240	27. 80
Bonne de Freuilles:				
Juice (Arlington).....	11. 34	. 330	. 328	13. 83
Whole fruit (Blacksburg).....	14. 52	. 560	. 510	21. 65
Bramtot:				
Juice (French).....	16. 00-16. 95	. 215-. 326	. 287-. 523	-----
Juice (Blacksburg).....	11. 01	. 343	. 610	12. 93
Juice (Arlington).....	15. 63-15. 94	. 187-. 316	. 338-. 460	17. 82
Whole fruit (Blacksburg).....	14. 52	. 560	. 510	21. 65
Julian de Paulmier:				
Juice (French).....	16. 90	. 190	. 330	-----
Juice (Arlington).....	11. 72-12. 90	. 182-. 350	. 326-. 610	15. 09-15. 61
Whole fruit (French).....	11. 86	. 330	. 160	22. 00
Whole fruit (Blacksburg).....	16. 54	. 218	. 380	26. 34
Launette Grosse:				
Juice (French).....	16. 07-16. 80	. 196-. 230	. 517-. 650	-----
Juice (Arlington).....	15. 16	. 229	. 686	17. 88
Whole fruit (French).....	14. 52	. 270	. 550	21. 50
Whole fruit (Blacksburg).....	10. 10-10. 70	. 415-. 574	. 230-. 480	19. 80-20
Maréchal:				
Juice (French).....	16. 80	. 260	. 970	-----
Whole fruit (French).....	13. 50	. 330	. 500	22. 2
Whole fruit (Blacksburg).....	12. 85	. 410	. 500	22. 4
Michelin:				
Juice (French).....	15. 92-16. 40	. 168-. 180	. 210-. 430	-----
Whole fruit (French).....	10. 92	. 32	. 220	19. 30
Whole fruit (Blacksburg).....	13. 05	. 725	. 290	23. 92
Montlige Blanc:				
Juice (French).....	15. 87	. 200	. 180	-----
Whole fruit (French).....	11. 60	. 290	. 210	19. 80
Whole fruit (Blacksburg).....	* 6. 43	. 164	. 160	17. 17
Moulin-à-Vent:				
Juice (French).....	16. 57-18. 00	. 198-. 290	. 350-. 365	-----
Juice (Blacksburg).....	11. 00	. 210	. 183	15. 77
Juice (Arlington).....	9. 77	. 163	. 405	11. 41
Whole fruit (French).....	12. 37	. 60	* 4. 40	21. 10
Whole fruit (Blacksburg).....	11. 27	. 410	. 410	20. 65

<sup>a</sup> Analyses of juices or whole fruits designated as "French" are quoted from Power (6), Truelle (8), or Warcollier (9), and are usually averages of 3 to 10 analyses. When two values are presented, they are extremes given by two or more of these authorities. Analyses of juices designated as "Arlington" or "Blacksburg" were made by the writer upon fruit grown at the location indicated. Analyses of whole fruit designated as "Blacksburg" were made by Price and Ellett (7).

<sup>b</sup> The values for acidity have been recalculated where necessary and are expressed as malic.

\* Analysis of fruit and juice at Blacksburg by Alwood, Davidson, and Moncre (2).

TABLE 2.—*Summary of analyses of juices and fruit of French-grown French cider apples and of French cider apples grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va.—Continued*

Variety and product	Total sugar	Acidity as malic	Total astringency	Solids
<i>Omout:</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Juice (French).....	14.20	0.33	0.260	-----
Juice (Arlington).....	10.37-15.62	0.164- .256	0.268- .640	21.13
Whole fruit (French).....	12.34	.37	.200	20.6
Whole fruit (Blacksburg).....	13.60	.232	.210	22.26
<i>Passe Reine:</i>				
Juice (Arlington).....	9.47	.988	.2984	11.20
Whole fruit (Blacksburg).....	13.02	.464	.760	22.55
<i>Petite Douce Rousse:</i>				
Juice (French).....	15.56	.270	.180	-----
Juice (Arlington).....	11.52	.202	.411	14.36
Whole fruit (French).....	11.30	.410	.140	21.00
Whole fruit (Blacksburg).....	9.65	.505	.470	d 29.80
<i>Reine des Pommes:</i>				
Juice (French).....	16.73-19.10	.184- .430	.268- .510	-----
Juice (Arlington).....	11.33	.189	.444	14.05
Juice (Blacksburg).....	11.90	.329	.594	13.80
Whole fruit (French).....	12.96	.570	.240	19.90
Whole fruit (Blacksburg).....	10.62-13.20	.428- .847	.340- .540	19.75-26.3
<i>Rouge du Landel:</i>				
Juice (French).....	14.12-15.07	.242- .230	.210- .283	-----
Juice (Blacksburg).....	10.00	.355	.2920	11.76
Juice (Arlington).....	9.68-10.49	.171- .296	.251- .292	11.93
Whole fruit (French).....	12.20	.31	.120	19.70
Whole fruit (Blacksburg).....	8.09	.53	.200	17.55
<i>St. Laurent:</i>				
Juice (French).....	15.50-15.90	.121- .340	.152- .340	-----
Whole fruit (French).....	13.88	.21	.210	25.50
Whole fruit (Blacksburg).....	16.04	.314	.270	28.30

<sup>d</sup> So in original; probably a typographical error.

The data thus assembled afford material for formulating an answer to the most important question with respect to the French cider apples, namely, whether the transfer from France to America has resulted in such fundamental changes in composition as to destroy their distinctive character as a group. This question may be definitely answered in the negative. As compared with the analyses of the same variety grown in France, the analyses of the fruit grown at Blacksburg or at Arlington show no large or consistent differences. The values for total sugar given by the French workers for juices are in practically every case somewhat higher than those found in the American work. The practically universal use of the saccharimeter instead of chemical determinations in the French studies of juices undoubtedly is responsible for the high values shown. It has repeatedly been demonstrated that the use of a saccharimeter with juices having a high content of nonsugar solids gives values materially in excess of those found by chemical determinations (3). That this is the real explanation of the high sugar content of French juices is made conclusive by the fact that the French determinations of sugar in the whole fruit, which must be made by chemical methods, agree quite well with the data on American-grown whole fruit. The disagreement between the French data on sugar in the whole fruit and in the juice of the variety is conclusive evidence that the juice figures are in excess of the true values.

In acid content the determinations upon French and American material, and particularly upon juices, show in general very close agreement. In tannin content the differences between the two

materials are greater, the American samples showing amounts sometimes larger, sometimes smaller than French fruit of the same variety, so that the differences do not indicate change in a definite direction. The differences between the samples grown at Arlington and at Blacksburg are in some cases greater than those between either and the samples grown in France.

Warcollier (9) and Truelle (8) collected data upon maximum, minimum, and mean composition of a number of French cider apples, based upon considerable numbers of analyses from various sources and from various regions of France. These show very wide ranges in amount of all constituents, as will be noted by reference to the examples reproduced from Warcollier in Table 3. There is a range of 100 per cent in sugar content in these analyses and of several times this amount in acid and tannin when the numbers of analyses are such as to suggest that the various soils and climatic conditions of France are adequately represented. The differences in composition of American-grown fruit from the French material analyzed by Power, Truelle, and Warcollier, as shown in Table 2, lie well within the extremes found in material from various parts of France.

TABLE 3.—*Maximum, minimum, and mean composition of juices of standard sorts of French cider apples*

[From Warcollier (9, p. 393)]

Variety	Number of analyses	Item	Total sugar	Acid	Tannin
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
St. Laurent.....	21	Maximum.....	24.31	0.730	0.699
		Minimum.....	12.63	.090	.096
		Mean.....	16.51	.276	.244
Bramtot.....	58	Maximum.....	26.35	.960	1.055
		Minimum.....	9.41	.085	.133
		Mean.....	19.05	.219	.529
Omont.....	3	Maximum.....	14.92	.370	.300
		Minimum.....	12.90	.310	.245
		Mean.....	14.19	.330	.266
Bedan des Parts.....	75	Maximum.....	21.94	.397	.825
		Minimum.....	10.68	.015	.008
		Mean.....	14.89	.140	.196

It may therefore be taken as rather definitely established that the French cider fruits, grown in Virginia at an elevation of 2,170 feet, as at Blacksburg, or only a few feet above sea level, as at Arlington, retain their distinctive characters without modification in any definite direction. All the apples here dealt with, with the exception of Passe Reine and Saint Hilaire, belong to the group called by the French "douce-amère" and by the English "bitter-sweet." They are characterized by a very high content of astringent materials and by an acidity lower than that of most of the American dessert varieties. The sugar content is not markedly higher than that of the better American dessert fruits, but is made to appear so to taste by the low acidity, while the high astringency and the persistent bitter aftertaste give them a very distinctive character.

It is believed that the combination of low acidity, high astringency, and medium to high sugar content found in these apples renders them potentially valuable material for blending with fruits of the dessert and culinary types in the making of unfermented juices and possibly

of other products. As is evident from their chemical composition, nearly all of the American cultivated apples are too low in astringency to furnish a juice of proper balance in its acid-astringency-sugar ratio. Winesap and Northern Spy are varieties which make acceptable beverage juices, primarily for the reason that their astringency at the proper pressing season is much higher than that of other commercial sorts. But it is not sufficiently high to permit extensive mixture of other sorts with them in cider making without upsetting the balance of the product in the direction of insipidity.

For use in blending with the usual types of apples for juice making, an ideal fruit would be one which could be added without disturbing the general level of sugar or acid content while bringing astringency up to the desired point.

The French cider apples thus far examined appear to be almost ideal material for meeting these requirements, in so far as the chemical characters of the fruit are concerned. It would seem a justifiable proceeding to give somewhat greater attention to these varieties to the end that their adaptability to varied conditions, relative susceptibility to the more important American diseases and pests, productiveness, and other pertinent horticultural data might be known. Data on the behavior of these apples such as Price and Ellett have given for the Blacksburg collection should be procured in other fruit-growing districts, since it would appear that some of these apples may have a definite place in American fruit growing.

#### SIBERIAN AND NATIVE CRABS AND OTHER ASTRINGENT APPLES

Table 4 presents analyses of 61 varieties of apples of widely varying origin and character, alike only in that they have a total content of astringent constituents equaling or exceeding 125 milligrams per 100 cubic centimeters. These may be roughly divided into groups on the basis of likeness with respect to other characters.

TABLE 4.—*Analyses of astrigent apples of various origins*

Variety	Date picked	Date analyzed	Constituents (per cent)								Acid- astrin- sugar ratio
			Reduc- ing sugar	Sucrose	Total sugar	Acid	Total astrin- gency	Tannin	Non- tan- nin	Solids	
Algérienne.....	(Aug. 19, 1922	Aug. 28	5.93	4.07	10.00	0.383	0.2761	0.1104	0.1657	13.60	1:0.72:27.6
	Sept. 13, 1923	Sept. 21	5.97	5.58	11.55	.221	.2500	.1315	.1185	12.97	1:1.13:52.2
	Aug. 10, 1924	Aug. 24	5.02	4.24	9.26	.231	.1930	.1008	.0922		1:1.04:79.6
	Oct. 7, 1925	Oct. 13	8.28	3.66	11.94	.150	.1570	.0812	.0758	14.29	1:0.26:23.7
Arctic.....	(Sept. 28, 1922	Dec. 8	8.26	2.15	10.41	.439	.1155	.0358	.0797		1:0.27:23
	Sept. 16, 1923	Nov. 15	4.84	4.84	13.97	.608	.1648	.0622	.1026	16.76	1:0.31:19.2
	Oct. 2	Oct. 2	3.38	3.38	10.10	.525	.1660	.0760	.0900		1:0.35:29
	Sept. 21, 1924	Sept. 26	8.62	1.84	10.46	.300	.1265	.0518	.0747	13.33	1:0.31:20.7
Augustine.....	Sept. 3, 1923	Sept. 18	10.42	3.82	14.24	.725	.2182	.0999	.1183	16.09	1:0.31:20.7
	Sept. 19, 1922	Aug. 9	6.04	2.80	8.84	.426	.1308	.0429	.0879	9.62	1:0.23:13.7
	July 30, 1923	July 30	6.04	4.30	10.34	.655	.1485	.0700	.0785	11.62	1:0.23:13.7
	(Sept. 23, 1922	Dec. 5	8.80	2.94	11.74	1.010	.1270	.0445	.0812	15.60	1:0.19:18.5
Canada Reinette.....	(Sept. 27, 1923	Oct. 4	7.38	4.79	12.17	.710	.1313	.0652	.0825	16.69	1:0.21:14.3
	Sept. 15, 1924	Oct. 6	7.10	3.08	10.18	.769	.1353	.0805	.0710	14.03	1:0.21:14.3
	Aug. 11, 1922	Aug. 14	8.46	2.39	10.85	.423	.1453	.0392	.0701	11.63	1:0.34:21.7
	Sept. 16, 1923	Nov. 22	6.08	3.16	9.24	.494	.1082	.0445	.0897	11.16	1:0.23:18.8
Colvert.....	(Sept. 13, 1924	Sept. 19	6.49	2.83	9.32	.474	.1082	.0392	.0600		1:0.287:18.5
	Aug. 12, 1922	Aug. 15	7.82	1.20	9.02	.346	.1482	.0406	.0817	12.55	1:0.287:18.5
	Sept. 3, 1923	Sept. 10	7.40	2.07	10.53	.430	.1353	.0798	.0846	13.35	1:0.43:31.3
	Aug. 20, 1924	Sept. 10	7.44	2.16	8.60	.424	.1300	.0585	.0845	9.72	1:0.31:21.7
Early Joe.....	July 20, 1922	Aug. 11	7.44	7.72	8.16	.352	.1742	.0792	.0807		1:0.33:20.2
	Aug. 10, 1924	Aug. 10	6.18	3.18	9.36	.733	.1375	.0735	.0850	11.20	1:0.49:23.1
	July 25, 1923	Aug. 30	6.26	2.46	8.72	1.117	.1460	.2313	.1147	16.04	1:0.38:8.9
	Sept. 18, 1922	Nov. 16	7.26	2.46	9.72	1.117	.1460	.2313	.1147	11.90	1:0.3:6.7
Early Strawberry.....	Sept. 25, 1923	Oct. 14	6.06	3.21	9.27	1.375	.1430	.2010	.2120	21.20	1:0.3:6.7
	Sept. 18, 1922	Nov. 16	5.26	2.94	8.20	1.425	.2880	.2347	.1483	11.47	1:0.38:8.9
	Sept. 25, 1924	Oct. 14	6.06	3.21	9.27	1.375	.1430	.2010	.2120	21.20	1:0.3:6.7
	Entz.....	Sept. 18, 1922	Nov. 16	5.26	2.94	8.20	1.425	.2880	.2347	.1483	11.90
Florence.....	Sept. 25, 1922	July 26	6.29	1.19	7.48	.836	.3229	.1441	.1788	9.30	1:0.3:6.7
	Aug. 9, 1924	Aug. 18	5.69	.60	9.12	.630	.2900	.1040	.1496		1:0.3:6.7
	July 20, 1925	Aug. 18	8.52	6.00	9.12	.620	.1940	.0942	.0908	9.30	1:0.47:11.9
	(Aug. 5, 1924	July 26	4.87	1.12	8.94	.612	.1410	.0597	.0813	7.62	1:0.31:14.7
Gladstone.....	Aug. 5, 1924	Aug. 8	7.22	1.12	8.94	.733	.1930	.0597	.1073		1:0.23:11.2
	July 31, 1920	Aug. 6	8.86	1.12	9.98	.144	.1280	.0460	.0820	11.40	1:0.23:11.2
	July 25, 1922	Aug. 7	8.37	1.20	9.57	.276	.1768	.0521	.1247		1:0.293:11.3
	Aug. 6	Aug. 6	8.09	3.43	9.52	.200	.1880	.0845	.0620	11.40	1:0.64:34.6
Golden Sweet.....	July 3, 1922	July 6	6.09	3.43	9.52	.200	.1880	.0845	.1035	10.72	1:0.94:47.6
	Aug. 3, 1922	Aug. 11	5.86	2.40	9.01	.979	.1327	.0565	.0762	7.62	1:0.13:9.2
	July 6, 1923	July 13	5.86	2.40	9.01	.979	.1327	.0565	.0801	13.21	1:0.14:7.7
	Sept. 3, 1923	Sept. 18	4.98	2.32	7.30	1.050	.1695	.1045	.0650	8.33	1:0.6:6.95

Hort. No. 4951 *	Sept. 24, 1920	Oct. 16	7.72	1.11	8.83	.960	.1470	.1190	11.20	1:0 27:9.1
	Sept. 16, 1921	Oct. 27	7.39	2.75	10.14	.901	.1340	.1450	-----	1:0 3:11.2
	Oct. 10, 1921	Nov. 17	7.46	.63	8.09	.915	.2924	.1494	-----	1:0 32:8.8
	Oct. 24, 1923	Nov. 8	7.33	2.16	9.49	.862	.2838	.1225	11.62	1:0 33:11
	Oct. 4, 1924	Oct. 16	6.37	2.16	8.51	1.140	.1815	.2095	-----	1:0 34:7.4
Hogg	Aug. 3, 1922	Aug. 4	6.80	2.71	9.51	1.454	.0828	.1185	13.14	1:0 14:6.5
	Aug. 14, 1923	Aug. 21	6.30	5.44	13.74	2.259	.0743	.0817	14.99	1:0 009:6.1
	Aug. 2, 1924	Sept. 15	6.11	3.93	10.04	1.066	.0690	.0672	-----	1:0 118:9.4
Hog Island	Sept. 2, 1922	Aug. 4	8.91	1.19	10.10	.212	.0460	.1461	12.76	1:0 9:47.6
	Aug. 27, 1923	Sept. 13	8.20	3.37	11.57	.177	.0553	.0728	12.48	1:0 7:65.3
	Aug. 30, 1922	Sept. 4	5.54	3.48	9.02	.583	.2077	.1207	11.62	1:0 56:15.4
	Sept. 8, 1923	Sept. 18	4.38	7.03	11.41	.517	.1768	.1412	12.79	1:0 61:22
Hyslop	Sept. 13, 1924	Sept. 22	3.37	6.21	9.58	.569	.3700	.3310	-----	1:0 65:16.8
	Sept. 13, 1924	Sept. 22	3.37	6.21	9.58	.569	.3700	.3310	-----	1:0 23:11.2
	Sept. 5, 1925	Sept. 14	3.44	5.92	9.36	.358	.2180	.0835	11.10	1:0 27:14.9
Irish Peach	July 14, 1922	Sept. 17	5.80	1.93	7.73	.520	.1390	.0879	-----	1:0 5:12.9
July	July 20, 1922	July 18	5.85	1.80	7.16	.552	.2730	.1210	-----	1:0 21:9.4
	July 23, 1922	July 25	6.48	1.08	8.16	.869	.1829	.0652	11.86	1:0 18:12.4
Jumbo	Aug. 2, 1923	Aug. 3	6.48	4.13	10.61	.852	.0777	.0808	11.82	1:0 58:11.2
	Aug. 2, 1923	Aug. 3	7.26	2.66	9.92	.880	.1645	.0795	11.99	1:0 58:11.2
	Aug. 23, 1922	Aug. 28	5.49	3.92	9.41	.688	.0639	.0709	13.80	1:0 74:30.7
Kennedy	Aug. 23, 1922	Aug. 28	8.89	1.00	9.89	.289	.2002	.0759	12.63	1:0 1:09.5
	Aug. 27, 1923	Sept. 12	5.31	3.53	11.04	.179	.1900	.0700	-----	1:0 82:65.2
Kentucky Sweet	Sept. 1, 1925	Sept. 27	7.66	2.38	10.04	.154	.0850	.0670	12.54	1:0 78:59.6
	Sept. 1, 1925	Sept. 27	6.74	2.14	8.85	.841	.1340	.0760	12.21	1:0 21:11.5
Lowland Raspberry	July 16, 1923	July 23	5.08	3.02	11.70	1.000	.0735	.1265	-----	1:0 26:16.9
	Aug. 5, 1924	Aug. 8	7.80	1.01	8.41	.687	.0594	.0832	-----	1:0 24:14.4
Labsk Reinette	July 13, 1923	Aug. 19	7.55	2.71	8.76	.687	.0594	.0832	15.88	1:0 76:16.9
	July 13, 1923	Aug. 19	7.55	2.71	8.76	.687	.0594	.0832	-----	1:0 84:23.2
	Aug. 19, 1923	Aug. 29	6.92	2.41	11.58	.687	.0594	.0832	-----	1:0 96:23
Lyman	Aug. 19, 1924	Sept. 22	8.67	3.20	11.90	.511	.2105	.2080	17.07	1:0 2:10.5
	Aug. 28, 1922	Sept. 5	9.98	2.47	12.46	.836	.2365	.2265	11.08	1:0 26:13.7
	Aug. 28, 1922	Sept. 5	9.98	2.47	12.46	.836	.2365	.2265	10.87	1:0 23:14.2
Martha	Aug. 6, 1922	Aug. 10	9.36	2.33	11.69	.850	.2820	.1027	14.41	1:0 34:30.2
	Aug. 15, 1922	Aug. 16	8.92	2.10	11.62	.824	.0925	.1015	-----	1:0 33:28.6
	Aug. 15, 1922	Aug. 16	8.92	2.10	11.62	.824	.0925	.1015	12.86	1:0 67:47.6
Muster	Sept. 5, 1924	Aug. 21	8.13	3.04	11.17	.390	.1310	.0655	13.09	1:0 36:33.3
	Sept. 5, 1924	Aug. 21	7.30	3.63	10.33	.217	.1452	.0427	14.36	1:1 10:80.3
Nain de Mahon	Sept. 16, 1923	Sept. 27	7.68	4.32	12.00	.360	.1322	.0949	-----	1:1 53:83.2
	Sept. 16, 1923	Sept. 27	7.68	4.32	12.00	.360	.1322	.0949	11.12	1:0 22:18.3
Nain de Mahon	July 30, 1922	Nov. 4	8.78	6.85	15.36	.172	.2050	.1090	-----	1:0 367:24.1
	July 31, 1922	Oct. 15	7.16	2.94	10.10	.304	.0796	.0841	12.09	1:0 72:42.4
Nain Paradis	Aug. 19, 1922	Oct. 25	8.30	2.99	11.20	.615	.1637	.0722	10.42	1:0 18:17.1
	Aug. 19, 1922	Oct. 25	7.12	2.88	9.50	.395	.0542	.0909	13.09	1:0 315:27.8
New England Pigeon	Sept. 8, 1923	Sept. 23	7.46	1.53	10.98	.212	.1525	.0972	-----	1:0 18:17.1
	Sept. 8, 1923	Sept. 23	8.12	2.86	10.98	.393	.1239	.0425	13.61	1:0 27:23.1
Northern Spy	Aug. 30, 1924	Oct. 8	6.54	3.16	9.70	.567	.1055	.0652	12.48	1:0 27:18.5
	Sept. 16, 1925	Aug. 18	8.06	3.32	11.38	.495	.1340	.0650	11.51	1:0 46:30.7
	Aug. 16, 1922	Aug. 18	6.36	4.42	10.78	1.055	.0899	.1022	-----	1:0 27:18.5
Peron	Aug. 23, 1923	Sept. 5	6.12	4.12	10.24	.680	.1012	.0681	12.43	1:0 46:30.7
	Sept. 5, 1924	Sept. 16	5.56	3.32	8.48	.458	.0400	.0762	-----	1:0 46:30.7
	Sept. 1, 1925	Sept. 8	5.56	4.06	9.62	.313	.1415	.0663	-----	1:0 46:30.7

\* The apple designated as Hort. No. 4951 was believed at the time of planting to be Golden (Golden Beauty). Its identity is in doubt, as it differs in some respects from the descriptions of that variety.

TABLE 4.—Analyses of astringent apples of various origins—Continued

Variety	Date picked	Date analyzed	Constituents (per cent)						Solids	Astringency-sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid	Total astringency	Tannin		
Piper.....	June 27, 1922	June 30	8.85	0.50	9.35	0.564	0.1614	0.0408	0.1206	1:0.28:16.5
	July 10, 1923	July 30	6.40	2.40	8.80	.580	.2180	.1010	10.67	1:0.37:15.1
	July 23, 1924	do.	5.86	3.56	9.42	.553	.1370	.0845	10.46	1:0.38:19.5
Primate.....	July 20, 1922	July 21	6.22	3.52	9.74	.608	.1287	.0849	10.94	1:0.27:20.4
	July 26, 1923	July 23	7.34	2.88	10.22	.574	.1385	.0887	10.97	1:0.16:11.1
	Aug. 1, 1923	Aug. 11	7.78	.38	8.16	.582	.0740	.0918	11.32	1:0.63:15.5
	Oct. 4, 1923	Oct. 11	2.68	3.22	5.90	2.316	.6882	.3380	9.02	1:0.09:1.30
	Oct. 26, 1923	Nov. 23	2.94	1.61	4.55	2.004	.6850	.4060	7.77	1:0.46:2.7
Pyrus angustifolia.....	Nov. 1, 1924	Nov. 1	2.97	1.47	4.44	2.604	.6900	.3270	10.40	1:0.27:1.77
	Nov. 4, 1925	Nov. 21	2.94	1.40	4.34	2.583	.6900	.3420	7.63	1:0.32:1.5
	Sept. 1, 1925	Sept. 5	9.63	2.15	11.78	2.583	.8350	.2930	8.13	1:0.32:44.2
	Sept. 13, 1924	Sept. 24	8.12	2.78	10.86	.118	.1605	.0675	13.25	1:1.36:92
Ramsdell Sweet.....	Oct. 7, 1925	Oct. 16	8.12	1.78	9.90	.078	.1460	.0475	12.10	1:1.87:127
	June 1, 1924	June 30	6.82	2.76	9.58	.960	.2095	.0787	11.63	1:0.21:9.9
Red Astrachan.....	July 14, 1923	July 18	7.46	3.21	10.67	1.380	.2550	.1180	13.70	1:0.18:7.7
Red Elser.....	Sept. 8, 1923	Sept. 19	7.64	4.04	12.68	.734	.1318	.0611	13.48	1:0.18:17.1
	July 15, 1922	July 17	6.01	1.84	7.85	.551	.1584	.0603	15.99	1:0.28:14.2
Red June.....	July 20, 1923	July 23	6.06	2.03	8.09	.686	.1373	.0713	10.56	1:0.21:13.6
	July 28, 1924	Aug. 13	6.54	2.43	8.97	.350	.1180	.0390	10.30	1:0.33:25.7
	Aug. 1, 1922	Aug. 7	9.24	4.06	13.30	1.351	.5283	.2340	16.36	1:0.30:9.8
Red Siberian.....	Sept. 3, 1923	Sept. 18	8.13	4.80	12.98	.638	.6600	.2280	16.22	1:0.7:13.8
	Sept. 10, 1924	Sept. 16	7.49	2.13	9.62	.605	.6450	.1465	14.10	1:0.9:15.9
Red Sweet.....	Sept. 3, 1923	Sept. 11	8.66	3.98	12.64	.175	.1740	.0800	14.10	1:0.99:72.2
	July 25, 1922	July 26	6.44	1.85	8.29	.769	.1685	.0399	10.72	1:0.21:10.7
Ruby Gem.....	Aug. 1, 1923	Sept. 12	6.00	4.94	10.94	.676	.1680	.0643	12.73	1:0.24:16.2
	Aug. 1, 1924	Aug. 30	5.66	1.84	7.50	.400	.1172	.0684	10.88	1:0.36:11.2
	Oct. 1, 1924	Oct. 16	5.89	1.39	7.28	.761	.1860	.0635	11.06	1:0.46:11.3
Souland Crab.....	Oct. 1, 1924	Oct. 16	5.89	2.36	8.25	.616	.2800	.1365	11.15	1:0.34:13.5
	Nov. 3, 1925	Nov. 25	6.18	2.36	8.54	.683	.2824	.1365	11.15	1:0.46:11.3
	Oct. 20, 1922	Oct. 24	7.45	2.73	10.18	.657	.1740	.0717	13.84	1:0.66:52.7
Springdale.....	Oct. 20, 1923	Oct. 31	8.44	4.28	12.72	.558	.1535	.0895	11.40	1:0.27:22.8
	Nov. 4, 1924	Dec. 2	7.70	1.66	9.36	.925	.1200	.0803	13.98	1:0.4:28.8
Stump.....	Aug. 24, 1923	Aug. 30	6.36	5.12	11.48	.534	.1300	.0625	12.63	1:0.24:21.5
Summer Pippin.....	July 25, 1923	do.	7.36	2.43	9.79	.792	.1500	.0765	11.39	1:0.19:12.8
Tetofski.....	Sept. 25, 1922	Nov. 13	5.10	2.38	7.48	.614	.1451	.0511	8.68	1:0.23:12.1
	Sept. 25, 1922	Nov. 13	7.52	2.80	10.32	.279	.1717	.0983	13.98	1:0.61:37.0
Titus Pippin.....	Sept. 25, 1923	Oct. 2	9.32	2.87	10.19	.582	.1182	.0625	13.14	1:0.2:17.5
	Oct. 1, 1925	Oct. 26	7.32	1.97	11.24	.314	.1390	.0630	14.74	1:0.44:35.7



Traders.....	July 28, 1922	July 31	6.87	2.01	8.88	.717	.1512	.0225	.1287	11.16	1:0.21:12.3
	Aug. 16, 1923	Aug. 24	8.34	4.92	13.26	.520	.2030	.1345	.0685	15.07	1:0.39:25.5
	Aug. 29, 1924	Sept. 4	6.51	2.61	9.17	.733	.1005	.0293	.0712	15.07	1:0.13:12.4
Transcendent.....	Aug. 1, 1922	Aug. 7	8.32	1.66	9.98	1.140	.3617	.1696	.1921	11.84	1:0.317:8.7
	Aug. 20, 1923	Aug. 27	7.44	4.27	11.71	.783	.2910	.1662	.1248	14.44	1:0.37:14.9
	Aug. 18, 1924	Aug. 21	7.64	1.16	8.80	.417	.3180	.1230	.1950	14.44	1:0.76:21.1
Trenton Early.....	Aug. 6, 1925	Aug. 10	6.41	3.68	10.09	.776	.2136	.1036	.1100	10.63	1:0.27:13
	July 10, 1922	July 17	6.68	1.74	8.42	.984	.1400	.0583	.0817	10.63	1:0.14:8.5
	Aug. 10, 1923	Aug. 13	6.02	1.08	7.10	1.027	.1725	.0623	.1102	11.79	1:0.168:6.9
Trumbull.....	Aug. 3, 1922	Aug. 7	9.18	3.75	10.19	.199	.1727	.0593	.1134	11.79	1:0.86:51.2
	Aug. 29, 1923	Sept. 5	6.86	3.02	12.93	.221	.1700	.0835	.0765	14.34	1:0.76:55.5
	Aug. 29, 1924	Sept. 12	7.04	3.16	9.88	.133	.1251	.0564	.0687	11.34	1:0.94:74.2
Whitney.....	July 26, 1922	July 26	10.40	4.42	10.20	.678	.1400	.0276	.1124	13.04	1:0.2:15
	Aug. 2, 1923	Oct. 25	6.67	4.10	14.82	.542	.1055	.0515	.0540	16.62	1:0.19:27.3
Williams.....	July 25, 1923	July 30	6.67	4.10	10.77	.551	.1420	.0695	.0725	12.82	1:0.25:19.5
	July 29, 1924	Aug. 6	6.67	2.13	8.82	.286	.1278	.0606	.0609	12.82	1:0.43:29.8
Wild Red.....	July 21, 1923	July 23	6.11	3.53	8.24	.357	.0556	.0311	.0545	11.36	1:0.22:21.3
	Nov. 1, 1924	Nov. 8	6.67	3.49	10.59	.723	.1700	.1369	.1301	12.04	1:0.43:13.9
	Oct. 18, 1923	Nov. 13	8.96	6.66	12.59	.143	.2245	.1325	.1340	12.04	1:0.38:17.3
Winter Paradise.....	Oct. 16, 1922	Oct. 18	8.76	1.26	9.96	.113	.1437	.0560	.0687	14.15	1:1.27:87.2
	Oct. 18, 1923	Oct. 25	7.02	3.34	12.18	.132	.1637	.0596	.0772	14.15	1:1.08:82.2
	Oct. 28, 1924	Nov. 1	8.84	2.40	9.42	.107	.1670	.0570	.0772	14.15	1:1.50:88.7
Worcester Pearmain.....	Oct. 10, 1925	Oct. 31	9.32	2.16	11.48	.092	.1620	.0870	.0947	13.84	1:1.15:14.7
	Aug. 1, 1922	Aug. 7	12.44	1.25	13.69	.328	.2930	.1470	.1400	13.84	1:0.38:10.0
Yellow Siberian.....	Sept. 6, 1923	Sept. 18	6.87	1.66	12.54	1.154	.3474	.1952	.2432	15.40	1:0.34:11.0
	Aug. 29, 1924	Sept. 19	7.82	4.53	11.90	1.010	.3760	.1982	.2432	13.02	1:0.63:18.5
	June 26, 1923	June 30	6.34	3.20	11.02	.593	.3760	.2335	.1425	9.24	1:0.28:11.6
Yellow Transparent.....	July 9, 1923	July 18	7.35	1.56	7.90	.680	.1942	.1125	.0817	9.24	1:0.16:6.7
	July 23, 1924	July 30	5.31	2.07	9.42	1.400	.2270	.0876	.1394	12.17	1:0.31:13.8
	July 11, 1925	July 17	5.92	1.61	7.53	.486	.1360	.0380	.0980	9.60	1:0.15:15.5
							.0753	.0255	.0498		

## ASTRINGENT FRUITS OF LOW ACID CONTENT

A relatively small group of varieties are comparable with the French cider-apple varieties previously discussed in that they are fairly high in sugar content while low in acidity, 0.5 per cent or less. In total astringency they as a class fall somewhat below most of the French cider group. Some of them have amounts exceeding those found in such French apples as Fréquin Lajoie, Fréquin Rouge, Précoce de Tunis, Rossignol, and Rouge du Landel, while others only slightly exceed 125 mgm. per 100 c. c. Examples of this class are Algérienne, Golden Sweet, Hog Island, Kentucky Sweet, Muster, Nain de Mahon, New England Pigeon, Ramsdell Sweet, Red Sweet, Trumbull, Winter Paradise, and Worcester Pearmain. By reason of their generally low acidity, they may be employed in combination with other apples of ordinary cultivated types for the same purpose as the French group, namely, the addition of astringency without material modification of sugar content, or for the addition of astringency together with the reduction of acid content. The fact that a few of them—for example, Golden Sweet and Ramsdell Sweet—are old varieties which have fairly wide distribution in home orchards and which are carried by nurserymen, may make them more easily obtainable than are the French apples of like acid-astringency-sugar ratios.

## ASTRINGENT FRUITS OF MEDIUM ACID CONTENT

A second group of the apples for which analyses are presented in Table 4 is made up of fruits whose juices have an acid content ranging from 0.5 to 0.75 per cent. This group includes Augustine, Arctic, Early Strawberry, Florence, Hyslop, Lyman, Soulard Crab, Whitney, Wild Red, and a number of others. It will be noted that a considerable number of the older and more widely known and cultivated crab apples are included in this list. The range of acidity in the group is somewhat above the general average of the juices of commercial varieties. Their function will be that of increasing both acidity and astringency of juices to which they are added. Some of the group, as Florence, Hyslop, Lyman, and Soulard Crab, have astringent contents comparable with those of the first group or with the French cider fruits, but most of the group are considerably below these in tannin content.

## HIGHLY ACID ASTRINGENT APPLES

A third group consists of apples which combine an acidity of 0.8 per cent or more with a considerable degree of astringency. Canada Reinette, Entz, Hogg, Jumbo, Martha, and Red Astrachan are typical of this group. Red Siberian and Yellow Siberian, while falling below the limit of 0.8 per cent acidity in one year (1924) are in normal years to be ranked with this group. In astringent content they present a rather wide range, Red Siberian, Yellow Siberian, and Entz having amounts comparable with the French apples, while Canada Reinette only slightly exceeds 125 mgm. per 100 c. c. In consequence the group contains material which can be employed to produce any desired degree of astringency and acidity in bulk material of subacid or sweet character. As several of the varieties of the group are old widely distributed apples which are grown both in home

orchards and in a limited commercial way, some one or another should prove to be adapted to cultivation in almost any of the important apple-producing districts.

Analytical data upon the juices of the wild Southern Crab, *Pyrus angustifolia*, have been included in the table for the purpose of calling attention to the very unusual character of the fruit of this species. Repeated analyses of the fruit of two trees on the Arlington farm grounds, continued over a series of years and checked by analyses on the fruit of the parent tree, are consistent in showing a remarkably low sugar content, an amount of astringent substances equaling or exceeding that of any of the French apples, and a titratable acidity approximating 2.5 per cent. The astringent material is somewhat suggestive of that of the French bitter-sweet group, having the bitterness characteristic of Amère du Surville and Julian de Paulmier. In amount of titratable acidity it very greatly exceeds any other apple thus far analyzed in this laboratory, having approximately twice the amount found in the most highly acid named varieties such as Entz, Hogg, Red Siberian, and Yellow Siberian. The fruit is of course quite inedible, but is occasionally gathered and used by housewives, small amounts being added to cultivated apples in making preserves or apple butter. The fruit keeps almost indefinitely in common storage and can be found under the fallen leaves late in March or April in perfectly sound condition and with acidity and astringency well preserved. Experiments with this apple have shown that its juice, added in very small quantities to such insipidly sweet juices as those of Delicious and other varieties lacking in both acid and tannin, greatly improves their palatability. In mixtures in which the quantity added does not exceed 3 to 5 per cent the bitterness which is so pronounced in the undiluted crab juice is subdued and becomes very agreeable, thus suggesting the effect of the bitter-sweet French apple juices.

#### SUMMARY

Attention has been called to the difficulties encountered in the manufacture of certain products from apples as a consequence of the prevailingly subacid and nonastringent character of the commercially popular varieties and of most of those widely grown in home orchards.

The necessity for giving more attention to varieties of highly acid and astringent character for blending with cultivated sorts in order to produce properly balanced products has been pointed out. As a contribution toward the information necessary as a guide in such work, analytical data have been presented upon 21 French cider-apple varieties grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va., and upon 61 astringent apple varieties of various origins grown at Arlington. That the various French cider apples undergo no very large or constant modification in their chemical character when grown either in the Virginia highlands at an elevation of 2,170 feet or a few feet above sea level at the Arlington farm is clear from comparisons of the analyses with the data from analyses made by French workers.

The 61 varieties of American, Siberian, and other apples which have been subjected to study may be grouped into three classes on

the basis of their titratable acidity as fruits of low, medium, and high acidity. These three groups will have somewhat different possibilities of usefulness. Each group contains varieties presenting a fairly wide range in content of astringent substances, as indicated by the range in acid-astringency-sugar ratios in each group. The varieties studied include most of the widely cultivated crab apples, together with others less widely known or confined to varietal collections. Taken collectively, the material discussed presents such a range of chemical character that it is possible to make selections adapted to meet almost any conceivable requirement of the manufacturer of apple products.

#### LITERATURE CITED

- (1) ALWOOD, W. B.  
1903. A STUDY OF CIDER MAKING IN FRANCE, GERMANY, AND ENGLAND, WITH COMMENTS AND COMPARISONS ON AMERICAN WORK. U. S. Dept. Agr., Bur. Chem. Bul. 71, 114 p., illus.
- (2) ——— DAVIDSON, R. J., and MONCURE, W. A. P.  
1904. THE CHEMICAL COMPOSITION OF APPLES AND CIDER. U. S. Dept. Agr., Bur. Chem. Bul. 88, 46 p.
- (3) CALDWELL, J. S.  
1925. SOME EFFECTS OF SEASONAL CONDITIONS UPON THE CHEMICAL COMPOSITION OF AMERICAN GRAPE JUICES. Jour. Agr. Research 30: 1133-1176, illus.
- (4) ———  
1928. CHEMICAL COMPOSITION OF APPLE JUICES AS AFFECTED BY CLIMATIC CONDITIONS. Jour. Agr. Research 36: 289-365.
- (5) ———  
1928. MEAN SUMMER OR "OPTIMUM" TEMPERATURES IN RELATION TO CHEMICAL COMPOSITION IN THE APPLE. Jour. Agr. Research 36: 367-389.
- (6) POWER, G.  
1884. TRAITÉ DE LA CULTURE DU POMMIER ET LA FABRICATION DU CIDRE. V. 2. MONOGRAPHIE DES MEILLEURES VARIÉTÉS DE POMMES À CIDRE. (Original not seen.)
- (7) PRICE, H. L., and ELLETT, W. B.  
1909. FIRST REPORT ON THE CULTURE AND ANALYSES OF FRENCH CIDER APPLES IN VIRGINIA. Va. Agr. Expt. Sta. Ann. Rpt. 1908: 39-54, illus.
- (8) TRUELLE, A.  
1896. ATLAS DES MEILLEURES VARIÉTÉS DE FRUITS À CIDRE. 88 p., illus. <sup>1</sup>  
Paris.
- (9) WARCOLLIER, G.  
1926. LE POMMIER À CIDRE ET LE POIRIER À POIRÉ. Ed. 3, 436 p., illus.  
Paris.

# CHEMICAL COMPOSITION OF THE JUICES OF SOME AMERICAN APPLES<sup>1</sup>

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## INTRODUCTION

The literature of American pomology is notably lacking in data upon the chemical composition of much of the material with which it deals. Such information as is available is largely derived from studies of a relatively small number of varieties of outstanding commercial importance. The focusing of attention upon these in connection with studies of development, ripening, and behavior in storage has resulted in the accumulation of fairly complete data in regard to their chemical composition. At the same time the study of varieties of lesser commercial importance has been neglected. Only a very small percentage of the 2,500 to 3,000 named varieties of apples recognized by authorities have ever been subjected to chemical analysis. The standard textbooks usually contain lists of apples recommended for home orchards or for commercial planting in specified districts, but for many of these no results of chemical analysis have been recorded. In consequence, the varietal descriptions found in the manuals, while giving such details of physical appearance and character of the various fruits as permit recognition by the student, can go no further in their attempts at chemical characterization than the use of the rather vague terms "subacid," "tart," "mildly acid," "sweet," and the like. Such descriptions, however adequate they may be for the purposes for which they are designed, are of little use when the fruit is considered as raw material to be converted into various preserved food products. The uses which may advantageously be made of a given variety for manufacturing purposes depend in some degree upon its physical character, but in much larger degree upon its chemical composition. In the absence of detailed chemical data upon more than a small number of varieties of apples, the manufacture of food products is rather rigidly confined to these known varieties. The necessity for the production of uniform, standardized products forbids random employment of unknown material, and in consequence a very large volume of potentially valuable raw material will remain unused until investigation of its chemical character yields data indicating its possibilities.

## REVIEW OF LITERATURE

Most of the analyses of apple juices made by American workers reported in the literature up to 1904 have been assembled by Van Slyke (12).<sup>2</sup> He reprinted analyses made by Browne (5) at the

<sup>1</sup> Received for publication July 28, 1926; issued May, 1928. This paper is the sixth in a series of studies on fruit juices.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 417.

Pennsylvania Agricultural Experiment Station, by Burd at Washington, D. C., for Alwood and his collaborators (1, 3), and by Davidson at the Virginia Agricultural Experiment Station (2), together with a number of analyses made at the New York Agricultural Experiment Station by Le Clerc and Van Slyke (12). His tables include data on 75 dessert or culinary apples and 10 crabs, and his paper would appear to contain the most extensive collection of analyses of American apples, either of whole fruit or of juices, to be found in the literature. Richards (11) published analyses of the whole fruit of about 18 varieties in 1887, and Browne (5) has made similar analyses of 25 varieties. Bigelow, Gore, and Howard (4) made a large number of analyses on whole fruits of a limited number of varieties at various stages of maturity, and Gore (9) published analyses of the fresh juices of 11 varieties. Jones and Colver (10) made very extensive analytical studies of the composition of whole fruits of about 30 varieties of apples, grown under both irrigated and unirrigated conditions, and in these studies they included all the more important commercial varieties grown in Idaho.

In the papers cited the analyses usually included such varieties as were rather extensively cultivated in the State or locality in which the work was done, and a number of varieties are common to most or all of the lists. The more recent literature contains a considerable amount of analytical data in regard to a relatively small number of varieties already included in the older papers, so that altogether not more than 200 varieties are represented in the literature by analyses either of the whole fruit or of the juices.

#### PURPOSE OF THE PRESENT WORK

One phase of the investigations having to do with the utilization of surplus and unmarketable horticultural products in progress in the fruit and vegetable utilization laboratory of the Office of Horticulture has had as its object the accumulation of data upon the chemical composition of apples with especial reference to the availability of the various varieties and varietal groups as material for the making of unfermented juices for beverage purposes. It was felt that such a chemical survey of a large mass of unknown material would have very considerable scientific interest and would at the same time be of practical value by calling attention to the availability for manufacturing purposes of a large volume of material not being used.

The plan pursued in the accumulation of such data was primarily one of annual analysis of the expressed juices of a large number of varieties growing under controlled conditions, for the purpose of procuring data as to composition, as to the range and character of the variations in composition occurring from year to year, and as to the relation of such variations to the annual fluctuations in seasonal climatic conditions. The results of such a study of more than 200 varieties of apples, continued over a period of six years, and of 60 varieties of grapes over a period of five years, have been published (6, 7).

With the twofold object of obtaining basic data upon the chemical composition of as large a number of apple varieties as possible and of discovering varieties of special characteristics which might make them valuable for special purposes, a large number of varieties have been

analyzed in this laboratory at various times since 1919. In a number of cases it was the original intention to make analyses annually in connection with the study to which reference has already been made, but the habit of irregular bearing, destruction of the crop by frosts, accidental injury or death of one of the trees, or limitation of time available for the work prevented this. In other cases no attempt was made to obtain analyses each year, but two analyses in different years were made when time permitted. Analyses of about 80 varieties of astringent apples are published elsewhere (8).

#### SOURCE OF MATERIAL

The fruit employed was grown in the apple variety collection of the Office of Horticulture, at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., except as otherwise noted, and consisted of sound tree-run samples of 1 to 5 bushels in quantity, of the mixed crop of the two trees of the variety. The samples were handled in all the details of storage, expression of juice, and analysis in the same manner as the material employed in the study already mentioned (7), which presents details as to the location of the orchard, character of soil, cultural treatment of the orchard, and methods of analysis employed. In so far as the joint judgment of two or three experienced persons could determine, all samples of fruit were pressed at a like stage of maturity, about halfway between picking-ripe condition and eating ripeness. In no case was an occasional crop subjected to analysis, that is, any pair of trees had in every case borne a crop approximating normal in amount in the year preceding that in which the crop was sampled for analysis.

#### SEASONAL CONDITIONS AS RELATED TO CHEMICAL COMPOSITION

The significance of the results of an isolated analysis of a variety of apple must remain questionable until much more is known as to the effect of latitude, climatic and soil conditions, culture, and fertilization upon the chemical composition of the fruit. In the absence of large numbers of analyses made at an identical stage of maturity upon a considerable number of varieties throughout the whole extent of their range and consequently under widely varying but measured conditions with respect to all the factors named, it can not be known to what extent an analysis of a variety grown at a given place and under a known set of conditions is indicative of what may be found under another set of conditions either at the same place or elsewhere.

Some background of information in regard to the extent to which variations in climatic conditions affect the composition of successive crops of the same trees is available for the analyses here presented. The six years 1920 to 1925, during which they were being accumulated, present several conditions approaching or equaling the maximum departures from normality recorded by the Washington station of the United States Weather Bureau during the 50-year period. While the year 1920 very closely approximated the normal in the amount and distribution of its rainfall, sunshine, and temperature, 1921 was the hottest year since 1891; 1923 had the largest number of hours of sunshine between March 1 and July 1 on record; 1924 had excessive precipitation with exceptionally low temperatures during the growing

season; and 1925 had a very large deficiency in spring and summer rainfall.

It has been shown elsewhere (7) that a large group of varieties upon which analyses were made during these years showed clear-cut mass responses to these differences in seasonal conditions. Employing the composition of the 1920 crop as a basis of comparison, the response consisted in an increase in sugar and acid content of the crop, accompanied by a decrease in astringent content, as the sunshine and degrees of temperature received during the growing season increased over the amounts received in 1920, and a decrease in sugar and acid and an increase in astringency as sunshine and temperature fell below those of 1920. In consequence of the control exerted over the chemical composition of the crop by the seasonal conditions, the crop of 1923 showed materially higher sugar content than did that of any other year, 1921 standing second, 1925 third, 1922 fourth, 1920 fifth, and 1924 sixth and lowest. High sugar content was accompanied by high acidity and low astringent content, low sugar content by low acidity and high astringent content, the comparative ranking of the crops of the various years with respect to each of these constituents being shown in Table 1.

TABLE 1.—Comparative rank in total sugar, titratable acidity, and relative astringency of apples analyzed in 1920–1925

Year	Total sugar <sup>a</sup>	Titrat-able acidity <sup>a</sup>	Relative astringency <sup>b</sup>	Year	Total sugar <sup>a</sup>	Titrat-able acidity <sup>a</sup>	Relative astringency <sup>b</sup>
1920.....	5	4	-----	1923.....	1	2	4
1921.....	2	1	-----	1924.....	6	5	1
1922.....	4	3	-----	1925.....	3	6	3

<sup>a</sup> Ranking of 1 indicates maximum; 6, minimum.

<sup>b</sup> Data upon relative astringency were not obtained in 1920 and 1921; hence the ranking in these items is from 1, the maximum, to 4, the minimum.

Since the material employed in the present analyses was grown in the same orchard in the same years and under the same conditions as that employed in the study from which the conclusions just stated were derived, it is a logical assumption that the fruit of the varieties here employed was affected in the same manner by the seasonal conditions. Consequently, analyses made in 1923 or 1921 will show a content of sugar which is high for the variety as grown in this orchard and which will be attained only under conditions exceptionally favorable for photosynthetic activity. Those made in 1924 or 1920 will show a minimum sugar content, reached only in years which are low in sunshine and temperature, while those made in 1922 or 1925 may be expected to present results near the middle of the range in composition of the variety as grown in this locality. The correlation of sugar content with acidity and astringency content, already mentioned, should also be found to obtain in these results.

The data of Table 2 contain material for testing the validity of these assumptions, since a considerable number of the varieties were analyzed in two or in three years of the period, in some cases in consecutive years, in others with an interval of one or two years between. Comparison of the results for the individual varieties, or of a group of varieties analyzed in the same years, will at once indicate whether the individual or the group behaved in accordance with the assumptions just made.



TABLE 2.—Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920-1924

Variety	Date of picking	Date analyzed	Constituents (per cent)						Total solids	Acid- strangency- sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid as malic	Total acid- strangency	Tannin		
Augustine.....	Sept. 3, 1923	Sept. 18	10.42	3.82	14.24	0.725	0.2182	0.0690	16.09	1:0.3:10.6
Banks Gravenstein.....	July 4, 1922	July 7	6.90	2.14	9.04	.875	.1144	.0071	10.88	1:0.13:10.3
Barbarie <sup>a</sup> .....	Aug. 7, 1923	Aug. 13	7.78	2.40	10.18	.715	.1117	.0518	11.82	1:0.15:14.2
Bedan des Parts <sup>a</sup> .....	Sept. 7, 1922	Oct. 21	10.10	1.54	11.64	.215	.3132	.1099	14.33	1:1.378:54.1
Berry.....	Aug. 28, 1922	Sept. 7	9.14	1.37	10.51	.146	.4070	.2745	11.40	1:2.75:54.1
Bigg.....	Sept. 23, 1922	Nov. 14	9.64	5.32	14.96	.314	.1168	.0478	0.690	1:0.372:47.6
Bloomfield.....	Sept. 16, 1923	Sept. 23	9.14	6.22	15.36	.332	.0990	.0340	15.97	1:0.298:46.2
Blue Mountain.....	Oct. 8, 1923	Oct. 23	6.96	3.88	10.84	.292	.0815	.0277	0.538	1:0.27:37.1
Bonne de Frenelles.....	Oct. 4, 1924	Oct. 14	6.11	3.63	9.74	.516	.0825	.0309	11.12	1:0.16:18.9
Bramfort.....	Aug. 23, 1922	Sept. 7	7.06	1.40	8.49	.703	.1104	.0371	12.27	1:0.187:12.1
Carlough.....	Aug. 14, 1922	Aug. 21	8.28	3.02	10.08	.812	.1070	.0552	11.98	1:0.146:13.7
Claus.....	Sept. 11, 1923	Sept. 5	6.08	2.40	10.68	.487	.0572	.0041	0.531	1:0.07:13.1
Clawis.....	Sept. 3, 1924	Sept. 22	6.09	3.25	9.33	.422	.0525	.0386	0.544	1:0.19:19.1
Connec.....	Sept. 11, 1922	Sept. 19	11.94	3.61	15.55	.330	.3280	.2050	13.83	1:0.12:20.7
Cornell.....	Sept. 6, 1923	Sept. 24	11.84	4.10	15.93	.316	.3385	.1735	16.50	1:0.09:34.3
Cox Golden.....	Oct. 20, 1922	Oct. 30	7.68	3.17	10.85	.285	.0956	.0363	17.82	1:1.07:40.4
Crookston.....	Nov. 14, 1924	Nov. 15	8.07	1.75	9.82	.223	.0498	.0194	13.01	1:2.46:83.2
D'Avrolles <sup>a</sup> .....	Oct. 4, 1923	Nov. 3	7.04	3.97	11.01	.371	.0735	.0406	18.00	1:0.22:44.3
D'Eve.....	Aug. 13, 1923	Aug. 25	7.50	4.12	11.62	.452	.0822	.0320	15.00	1:0.27:37.1
Drop de 'Or.....	Sept. 8, 1922	Sept. 16	8.54	2.00	10.54	.440	.0814	.0406	12.95	1:0.18:20.8
Dulaney.....	Sept. 5, 1923	Sept. 13	6.70	5.50	12.20	.136	.1750	.0882	15.75	1:0.25:07.5
Dumelow.....	Aug. 29, 1922	Aug. 4	7.96	5.50	13.46	.600	.1020	.0382	11.78	1:0.816:98.9
Early Edward.....	Sept. 3, 1922	Sept. 8	6.96	2.25	9.21	.348	.1017	.0163	11.78	1:0.17:15.7
.....	Aug. 29, 1924	Sept. 20	6.90	3.02	9.92	.406	.0911	.0385	12.70	1:0.29:25.8
.....	Sept. 18, 1922	Nov. 13	7.94	2.68	10.62	.280	.1115	.0178	0.633	1:0.163:20
.....	Oct. 6, 1922	Oct. 19	7.08	3.08	10.16	.756	.0615	.0208	12.45	1:0.38:36.7
.....	Oct. 21, 1923	Oct. 31	7.82	3.51	11.33	.715	.0702	.0205	0.497	1:0.08:13.4
.....	Oct. 18, 1924	Nov. 20	8.55	3.21	11.76	.840	.0462	.0255	0.007	1:0.09:15.8
.....	Oct. 4, 1922	Oct. 18	11.53	1.83	13.36	.210	.0402	.0255	14.68	1:0.197:63.6
.....	Oct. 7, 1922	Oct. 20	8.54	2.02	10.56	.261	.1221	.0558	13.38	1:0.47:40.4
.....	Nov. 4, 1924	Nov. 27	8.36	2.14	10.50	.213	.1125	.0617	0.608	1:0.52:46.3
.....	Sept. 14, 1923	Nov. 12	8.50	4.06	12.56	.770	.1090	.0615	15.92	1:0.142:16.3
.....	Aug. 10, 1922	Aug. 16	8.54	2.40	11.00	.525	.0960	.0204	11.98	1:0.18:20.9
.....	Oct. 14, 1921	Oct. 30	6.63	4.73	11.36	.531	.0680	.0223	13.83	1:0.184:21.4
.....	Aug. 29, 1922	Aug. 29	8.33	1.15	9.48	1.116	.1726	.0587	12.93	1:0.154:3.5
.....	Sept. 16, 1923	Sept. 25	9.80	2.59	12.45	.957	.1160	.0510	14.55	1:0.121:13.0
.....	July 20, 1922	July 28	7.64	.56	8.20	.581	.1573	.0443	11.70	1:0.27:14.1
.....	July 30, 1923	July 30	6.78	2.58	9.36	.821	.1200	.0585	11.41	1:0.146:11.4

<sup>a</sup> Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

TABLE 2.—Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920-1924—Continued

Variety	Date of picking	Date analyzed	Constituents (per cent)						Acid- astri- ngency- sugar ratio		
			Reduc- ing sugar	Sucrose	Total sugar	Acid as malic	Total astri- ngency	Tannin		Non- tannins	Total solids
Elstun.....	{Oct. 8, 1923 Oct. 4, 1924	{Oct. 10 Sept. 16	7.72 6.91	4.24 3.89	11.96 10.80	0.337 .212	0.0815 .0881	0.0200 .0552	.0615 .0532	14.06	1:0.24:35.4 1:0.41:50.9
Ensee.....	{Oct. 17, 1921 Sept. 10, 1923	{Sept. 6 Aug. 18	7.80 8.40	2.80 5.17	10.60 13.57	1.070 .715	.0516 .0947	.0010 .0612	.0506 .0605	12.44 14.94	1:0.05:9.9 1:0.132:18.9
Excelsior.....	{Oct. 9, 1920 Aug. 9, 1923	{Oct. 28 Aug. 17	7.70 8.04	1.51 4.34	9.21 12.38	.685 .417	.0614 .0831	.0180 .0300	.0734 .0531	13.21 13.21	1:0.133:13.4 1:0.2:29.6
Fall Cheese.....	{Oct. 3, 1922 Aug. 20, 1923	{Aug. 4 Sept. 27	7.35 6.20	3.34 4.45	10.69 10.74	.816 .478	.1287 .1655	.0562 .0555	.0695 .0500	11.94 11.88	1:0.16:13.1 1:0.22:22.1
Fall Swaar.....	{Oct. 12, 1922 Oct. 12, 1923	{Dec. 6 Nov. 22	9.59 10.06	2.03 2.46	12.22 12.52	.378 .291	.0773 .1072	.0303 .0180	.0570 .0592	15.17 13.34	1:0.2:29.6 1:0.368:43
Ferdinand.....	{Sept. 23, 1924 Oct. 8, 1923	{Oct. 17 Oct. 10	7.18 7.73	3.89 3.23	11.07 11.02	.630 .852	.1013 .2780	.0293 .0600	.0720 .0780	13.34	1:0.16:17.6 1:0.133:12.9
Footle.....	{Oct. 28, 1923 Aug. 28, 1923	{Oct. 10 Sept. 10	7.49 7.40	2.89 4.05	10.38 13.76	.318 .370	.1265 .1568	.1515 .0568	.1265 .1000	11.66 15.68	1:0.87:32.3 1:0.42:37.2
Fréquin Lajoie *.....	{Sept. 16, 1923 Oct. 10, 1922	{Sept. 29 Oct. 11	9.71 8.29	4.05 4.04	13.76 12.33	.370 .697	.1113 .1170	.0368 .0455	.0745 .0515	12.95	1:0.16:17.6 1:0.21:20.5
Fréquin Rouge.....	{Sept. 17, 1924 Sept. 10, 1923	{Nov. 20 Aug. 17	7.44 7.54	3.90 3.34	11.34 12.88	.652 .458	.1170 .1050	.0455 .0655	.0715 .0505	14.88	1:0.21:20.5 1:0.229:28.1
Gasconne.....	{Aug. 14, 1922 Aug. 14, 1923	{July 25 Aug. 8	8.64 9.48	3.75 1.08	12.39 8.10	.128 .889	.1000 .1529	.0113 .0662	.0387 .1297	8.24 11.86	1:0.78:57.7 1:0.21:9.4
Gent Sweet.....	{Aug. 20, 1922 Aug. 12, 1923	{July 26 Aug. 31	7.22 8.08	1.12 3.11	8.34 11.23	.733 .131	.1733 .0674	.0857 .0194	.1073 .0480	13.40	1:0.293:11.3 1:0.39:9.9
Gladstone.....	{Aug. 6, 1924 Aug. 13, 1923	{Aug. 8 Aug. 31	6.61 5.86	4.52 2.40	11.13 6.91	.958 .804	.0720 .1321	.0342 .0595	.0378 .0602	10.91	1:0.14:9.2 1:0.144:7.7
Golden Noble.....	{Aug. 12, 1922 Aug. 10, 1922	{Aug. 30 Aug. 25	5.06 7.15	4.40 2.53	9.46 9.68	.591 .392	.0929 .0850	.0320 .0250	.0820 .0600	12.80	1:0.154:15.8 1:0.217:28.2
Grand Sultan.....	{July 6, 1923 Aug. 8, 1923	{July 13 Aug. 26	5.86 7.43	1.05 3.86	6.91 10.30	.804 .393	.1321 .1010	.0520 .0293	.0801 .0725	12.50	1:0.15:22.8 1:0.27:28.3
Hackman.....	{Oct. 8, 1923 Oct. 2, 1924	{Oct. 18 Sept. 11	7.03 6.37	3.62 1.95	10.65 8.32	.478 .100	.0717 .1018	.0436 .0755	.0574 .0550	12.15	1:0.15:22.8 1:1.38:88.5
Harwell.....	{Sept. 3, 1921 Oct. 2, 1924	{Oct. 18 Sept. 11	7.08 6.37	2.62 1.80	9.70 8.16	.364 .169	.0717 .1018	.0436 .0755	.0574 .0550	12.15	1:0.15:22.8 1:1.38:88.5
Haskell.....	{Aug. 24, 1920 Sept. 16, 1924	{Sept. 11 Se- t. 24	7.14 6.37	1.80 1.95	8.94 8.32	.100 .004	.1305 .1066	.0405 .1045	.0650 .1461	8.33	1:0.161:6.95 1:0.906:47.6
Hewes.....	{Sept. 16, 1924 Sept. 3, 1922	{Se- t. 24 Aug. 4	4.86 8.20	2.32 3.37	7.18 10.57	.212 .702	.1921 .0961	.1045 .0337	.1461 .0788	12.70	1:0.712:65.3 1:0.125:12.6
Hog Island.....	{Aug. 27, 1923 Aug. 3, 1922	{Sept. 4 Aug. 8	8.20 7.84	2.16 2.98	10.36 10.82	.702 .303	.0961 .1096	.0337 .0205	.0788 .0632	11.91	1:0.32:38.3 1:0.194:48.5
Hope.....	{Aug. 7, 1922 Aug. 23, 1922	{Aug. 8 Sept. 29	7.22 6.22	2.42 2.08	9.64 8.30	.219 .272	.1010 .0456	.0205 .0821	.0222 .0135	12.47	1:0.16:35.7 1:0.32:30.8
Isban.....	{Aug. 23, 1922 Sept. 28, 1923	{Aug. 29 Dec. 22	8.22 7.18	2.42 4.93	10.64 12.11	.330 .219	.1070 .0427	.0455 .0821	.0615 .0135	12.47	1:0.16:35.7 1:0.33:29
Jacob Sweet.....	{Sept. 28, 1923 Aug. 22, 1924	{Dec. 22 Aug. 27	8.05 7.49	2.11 2.23	10.16 9.72	.330 .272	.1070 .0456	.0455 .0821	.0615 .0135	12.47	1:0.16:35.7 1:0.33:29
Jenks.....	{Aug. 22, 1924 Aug. 16, 1922	{Aug. 27 Aug. 17	9.24 8.71	2.23 2.05	11.46 10.76	.370 .745	.1266 .0952	.0560 .0282	.1206 .0905	12.60 12.79	1:0.473:44.4 1:0.128:16.6
Jerusalem Sweet.....	{Aug. 27, 1923 Nov. 4, 1923	{Sept. 24 Oct. 5	8.20 7.01	4.17 2.50	12.37 9.51	.745 .895	.0952 .895	.0282 .0403	.0670 .0712	13.78	1:0.128:16.6 1:0.138:11.8
John Sharp.....	{Oct. 4, 1924	{Oct. 15	7.01	2.50	9.51	.895	.895	.0403	.0712	13.78	1:0.138:11.8

	Aug. 23, 1923	Sept. 12	8.94	1.24	10.18	.404	.1290	.0640	.0650	11.17	1:0.32:25.2
Jones's Green.....	{Sept. 3, 1923	Oct. 6	7.80	1.09	9.46	.517	.0910	.0370	.0650	11.17	1:0.32:25.2
Jourveaux <sup>a</sup> .....	{Sept. 2, 1923	Sept. 7	9.07	1.44	11.14	.178	.3480	.2215	.1265	14.10	1:0.174:18.3
July.....	{Sept. 20, 1922	July 26	4.67	1.30	7.84	.618	.6140	.0638	.0648	17.82	1:1.95:02.5
Jumbo.....	{July 16, 1923	July 23	7.26	2.06	9.92	.953	.880	.0777	.0795	11.84	1:0.092:17.2
Kennedy.....	{July 21, 1923	July 30	5.49	2.92	6.41	.568	.1545	.0745	.0705	11.05	1:0.175:14.4
Kentucky Long Stem.....	{Oct. 16, 1923	Oct. 19	8.30	4.16	12.46	.450	.0986	.0659	.0709	10.80	1:0.24:16.5
Kittagee.....	{Oct. 1, 1924	Nov. 8	7.14	3.10	10.30	.447	.1130	.0582	.0745	14.86	1:0.22:27.6
Kittagee.....	{Sept. 27, 1923	Sept. 25	8.92	4.23	13.15	.587	.0870	.0283	.0587	14.22	1:0.25:23.0
Launette <sup>a</sup> .....	{Sept. 15, 1924	Oct. 18	8.70	3.08	11.78	.570	.1057	.0845	.0712	11.93	1:0.18:20.6
Do.....	{Sept. 1, 1923	Sept. 7	8.55	3.08	11.63	.220	.5025	.2805	.2160	11.93	1:2.19:50.8
Launette Grosse.....	{Sept. 15, 1923	Sept. 24	9.43	3.18	10.64	.219	.4700	.2450	.2250	11.93	1:2.29:50.7
Lowland Raspberry.....	{July 10, 1923	Sept. 18	11.98	3.02	15.16	.269	.6865	.3780	.3780	17.88	1:0.29:12.3
Lowland Raspberry.....	{Aug. 5, 1924	Aug. 8	7.80	.01	8.41	.682	.2000	.1132	.1265	12.21	1:0.20:12.3
Loddington.....	{Aug. 14, 1924	Aug. 10	6.85	2.85	9.70	.1048	.0671	.0734	.0797	10.04	1:0.092:9.2
Loy.....	{Sept. 27, 1923	Oct. 9	7.18	2.74	9.92	.1065	.1132	.0632	.0632	10.36	1:0.106:23
Lubsk Reihette.....	{Sept. 28, 1924	Oct. 20	6.60	3.10	9.70	.520	.0625	.0625	.0630	13.43	1:0.085:14.8
Lucinda.....	{July 10, 1923	July 19	7.55	1.47	9.02	.533	.1432	.0594	.0594	10.62	1:0.12:18.6
Lubsk Reihette.....	{Sept. 16, 1923	Sept. 27	6.64	2.74	8.76	.607	.0720	.0697	.0632	11.94	1:0.24:14.4
Magnet.....	{Oct. 8, 1924	Oct. 10	6.65	3.81	10.45	.482	.0970	.0430	.0540	10.62	1:0.107:15.6
Mason Orange.....	{Sept. 8, 1923	Sept. 22	7.34	3.76	11.10	.343	.0694	.0578	.0578	12.74	1:0.21:21.5
McCord.....	{Oct. 8, 1923	Nov. 5	8.18	3.78	10.91	.294	.0853	.0422	.0583	13.92	1:0.37:32.3
Melo Annurco.....	{Oct. 4, 1924	Oct. 11	7.72	3.80	11.02	.427	.0774	.0206	.0514	18.92	1:0.25:28.1
Melo Lemoncelli.....	{Oct. 8, 1923	Oct. 19	6.64	3.01	9.65	.426	.0868	.0652	.0756	12.22	1:0.19:22.6
Moulin-à-Vent <sup>a</sup> .....	{Oct. 12, 1923	Oct. 11	8.30	1.83	9.50	.510	.1110	.0430	.0680	16.33	1:0.21:18.6
Nain de Mahon.....	{Oct. 28, 1923	Nov. 20	6.93	2.37	9.30	.466	.0625	.0241	.0684	16.33	1:0.2:28.6
Nain de Mahon.....	{Oct. 9, 1923	Oct. 17	7.40	2.51	12.02	.612	.1055	.0607	.0573	15.88	1:0.35:30.3
New England Pigeon.....	{Sept. 15, 1923	Sept. 27	7.68	4.22	12.90	.163	.0600	.0340	.0688	11.41	1:0.17:20.6
New England Pigeon.....	{Sept. 15, 1923	Nov. 4	7.12	6.93	15.86	.172	.2650	.1322	.1720	14.36	1:2.48:60
Nonesuch.....	{Aug. 8, 1923	Sept. 23	6.87	2.88	8.30	.395	.0949	.0640	.0680	12.09	1:0.307:33.3
Omont.....	{Aug. 21, 1924	Aug. 24	4.49	1.63	6.46	.173	.1451	.0972	.0772	10.42	1:1.17:29.1
Omont.....	{Sept. 11, 1923	Sept. 18	8.50	1.83	9.25	.650	.1205	.0308	.0636	11.42	1:0.77:42.4
Palouse.....	{Oct. 1, 1922	Oct. 14	8.02	3.45	15.62	.296	.6400	.3290	.3110	21.13	1:0.185:9.6
Par.....	{Oct. 1, 1922	Aug. 17	8.18	2.60	10.37	.864	.2680	.1060	.1620	11.64	1:2.5:61
Parry White.....	{Nov. 4, 1923	Nov. 13	7.64	2.44	11.62	.264	.0845	.0100	.0745	13.66	1:1.63:95
Pette Drouce Rousse.....	{Aug. 16, 1924	Aug. 21	6.90	2.60	10.24	.800	.0735	.0254	.0481	13.66	1:0.26:39.8
Pette Drouce Rousse.....	{July 13, 1922	Sept. 28	7.86	1.61	9.47	.988	.1035	.0481	.0594	10.51	1:0.27:38.7
Plot (seedless).....	{Aug. 7, 1922	Aug. 8	8.20	3.32	11.52	.202	.2984	.1339	.1045	11.90	1:0.129:11.3
Plot (seedless).....	{Aug. 31, 1923	Sept. 4	6.29	4.05	10.34	.770	.4110	.2628	.1482	14.36	1:0.3:0.58
Plot (seedless).....	{Aug. 31, 1923	Sept. 4	6.29	4.05	10.34	.770	.4110	.2628	.1482	11.78	1:2.03:57
Plot (seedless).....	{Aug. 31, 1923	Sept. 4	6.29	4.05	10.34	.770	.4110	.2628	.1482	10.34	1:0.140:8.56
Plot (seedless).....	{Aug. 31, 1923	Sept. 4	6.29	4.05	10.34	.770	.4110	.2628	.1482	10.34	1:0.12:13.43

<sup>a</sup> Slightly past prime condition for pressing when received at the laboratory.

<sup>a</sup> Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

TABLE 2.—Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920-1924—Continued

Variety	Date of picking	Date analyzed	Constituents (per cent)						Tannin	Non-tannins	Total solids	Acid- stringency- sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid as malic	Total stringency					
Rambour Pappeau.....	Sept. 19, 1923	Sept. 24	6.48	3.26	9.74	0.156	0.1355	0.0343	0.0692	11.06	1:0.8:62.4	
.....	Sept. 13, 1924	Oct. 6	7.38	2.54	9.92	.253	.0627	.0217	.0710	.....	1:0.366:50.2	
Randolph.....	July 18, 1923	July 23	6.64	3.50	10.14	.632	.1215	.0711	.0894	10.08	1:0.16:16.16	
.....	Aug. 4, 1924	Aug. 7	7.40	2.27	9.67	.434	.1170	.0608	.0862	.....	1:2.7:22.3	
Red Eiser.....	Sept. 4, 1923	Sept. 10	8.94	4.94	12.88	.734	.1318	.2835	.0847	15.09	1:0.7:17.17	
Remo des Pommés.....	Sept. 17, 1923	Sept. 22	8.15	3.18	11.33	.399	.0845	.2835	.0862	14.05	1:2.34:60.0	
Rossignol.....	Sept. 17, 1923	Sept. 22	8.15	3.18	11.33	.399	.0845	.2835	.0862	13.80	1:1.8:36.0	
Rouge du Landel.....	Sept. 16, 1922	Oct. 21	10.23	1.99	12.22	.332	.0882	.0956	.1026	15.37	1:0.06:36.8	
Do.....	Sept. 16, 1922	Sept. 21	8.20	1.80	10.00	.355	.2620	.1432	.1498	11.70	1:0.823:28.2	
.....	Sept. 7, 1923	Sept. 20	8.23	1.45	9.68	.296	.2512	.1002	.1510	11.93	1:0.848:32.7	
Shannon.....	Sept. 7, 1924	Sept. 15	7.58	2.91	10.49	.171	.2620	.1575	.1345	.....	1:1.7:61.3	
Smith Early Red.....	Oct. 3, 1924	Oct. 14	6.98	3.75	10.33	.446	.0675	.0245	.0430	.....	1:0.15:23.1	
Stump.....	Sept. 13, 1924	Sept. 27	5.74	5.12	9.14	.575	.0755	.0250	.0505	11.22	1:0.13:15.9	
.....	Aug. 23, 1923	Aug. 30	6.36	5.12	11.48	.584	.1300	.0255	.0675	12.63	1:0.24:21.5	
Summer Extra.....	July 26, 1922	July 27	7.76	1.53	9.29	.645	.1430	.0296	.1134	11.04	1:0.18:17.6	
Summer Pippin.....	Aug. 13, 1923	Aug. 17	8.28	3.29	11.57	.655	.1187	.0467	.0720	13.34	1:0.19:12.3	
Sweet Fameuse.....	July 25, 1923	Aug. 30	7.36	2.43	9.79	.792	.1600	.0765	.0735	11.39	1:0.19:12.3	
.....	Sept. 16, 1923	Dec. 5	9.13	2.55	11.68	.164	.1200	.0713	.0857	14.68	1:0.73:71.2	
Taupaki.....	Sept. 15, 1924	Oct. 2	6.20	1.89	8.09	.225	.0906	.0498	.0408	.....	1:0.44:32.0	
.....	Aug. 29, 1922	Sept. 5	8.96	1.20	10.16	.680	.0869	.0543	.0526	12.66	1:0.13:14.9	
Tidal.....	Oct. 12, 1923	Oct. 22	6.90	3.21	10.11	.686	.1132	.0157	.0675	11.96	1:0.16:14.7	
.....	Oct. 18, 1924	Oct. 23	7.00	2.36	9.36	.288	.0806	.0334	.0472	11.13	1:0.27:36.9	
Townsend.....	July 21, 1922	July 31	6.26	2.95	9.21	.740	.0899	.0235	.0235	10.47	1:0.025:32.5	
.....	Aug. 3, 1923	Aug. 13	7.06	3.37	10.43	.462	.0855	.0342	.0513	11.61	1:0.15:12.4	
Trenton Early.....	July 10, 1922	July 17	6.68	1.74	8.42	.984	.1400	.0583	.0817	10.63	1:0.18:22.5	
.....	Aug. 10, 1923	Aug. 13	6.02	1.08	7.10	.027	.1725	.0324	.1102	.....	1:0.142:8.5	
Vanhoy.....	Oct. 26, 1923	Oct. 31	6.40	5.83	12.23	.607	.0924	.0623	.0600	12.72	1:0.168:6.9	
.....	Oct. 28, 1924	Nov. 11	7.06	2.72	9.77	.695	.0965	.0415	.0490	.....	1:0.15:20.1	
Whinery.....	Oct. 6, 1922	Oct. 30	8.00	3.00	11.00	.228	.0952	.0282	.0170	12.85	1:0.43:57.2	
Whitney.....	Oct. 12, 1923	Oct. 26	7.64	3.36	11.00	.286	.0642	.0230	.0412	12.04	1:0.2:1.6	
.....	July 26, 1922	July 26	10.40	4.42	14.82	.673	.1349	.0515	.0515	13.04	1:0.10:27.3	
William Anderson.....	Aug. 2, 1923	Nov. 24	5.86	2.62	8.48	.580	.1092	.0511	.0581	14.02	1:0.19:27.3	
.....	Sept. 15, 1923	Nov. 24	5.86	2.62	8.48	.580	.1092	.0511	.0581	14.02	1:0.19:27.3	
Wisner.....	Sept. 22, 1924	Sept. 27	8.04	3.11	11.15	.328	.1068	.0488	.0630	12.94	1:0.188:19.9	
Worcester Pearmain.....	Sept. 19, 1923	Nov. 27	8.04	3.11	11.15	.328	.1068	.0470	.0698	14.85	1:0.44:1.7	
Yellow Pippin.....	Aug. 14, 1923	Aug. 17	12.44	1.25	13.69	.297	.2030	.1470	.1460	.....	1:0.89:41.1	
.....	Nov. 4, 1924	Dec. 2	7.53	2.53	10.06	.317	.1180	.0412	.0768	.....	1:0.37:32.3	
Yellow Skin.....	Oct. 11, 1922	Oct. 12	6.85	4.62	11.47	.558	.0754	.0254	.0500	.....	1:0.135:20.5	
.....	Oct. 18, 1924	Oct. 23	7.99	2.34	10.33	.475	.0785	.0297	.0488	.....	1:0.165:21.7	

\* Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

\* Slightly past prime condition for pressing when received at the laboratory.

That these assumptions hold in a general way is apparent from examination of the data. Fifty-two varieties were analyzed in 1923 and also in some other year (in two instances in two other years). In 40 of these, the 1923 analyses showed higher sugar content than did the other years. In 27 of the 52, total acidity is higher in 1923, and in 33 relative astringency is lower than in the other year or years. This is in good agreement with the results obtained with the group analyzed annually, for which 1923 was the year of maximum sugar and minimum relative astringency content. The year of minimum sugar content in the work with the large group was 1924. Thirty-eight varieties of this group were analyzed in that year and also in some other year or years. Thirty-two of these had lower sugar content, 25 had lower acid content, and 25 had higher relative astringency in 1924 than in the other year in which they were analyzed. Thirty-six varieties were analyzed in 1922 and also in some other year. In 26 of these cases there was also an analysis made in 1923, a year of maximum sugar content in the large group. In 18 of these 26 varieties sugar content was lower, and in 19 relative astringency was higher, in 1922 than in 1923. Twelve of the varieties analyzed in 1922 were also analyzed in 1924, which was in the case of the large group a year of lower sugar content than was 1922. In 9 of these the sugar content was higher, in 10 the acid content was higher, and in 10 the relative astringency was lower in 1922 than in 1924.

The analyses of 1922 and 1923 present one exception to the situation generally present, in that the acid content of the 1922 samples is not prevalingly lower than in the same varieties in 1923. While actually lower in 10 of the 26 cases, it is equal or higher in the remaining 16. Acid content thus fails to behave in agreement with sugar content in this instance. This emphasizes the fact that climatic conditions influence sugar and acidity in the same direction but not necessarily in the same degree; there is no relationship between the two constituents such that they must necessarily rise or fall strictly together.

With this exception, the results of the analyses conform, in the quantity and interrelations of the constituents under discussion, to expectations based upon the group studied consecutively over the period. The exceptions discoverable would appear to be of the frequency and character observable in all work involving large numbers of individuals, whether of one variety or of various botanical or horticultural varieties. It therefore appears to be justifiable to consider that any analysis here presented has somewhat greater meaning than can be attached to an isolated analysis unaccompanied by any information as to the conditions under which the material employed was grown. The years in which the analyses were made have been evaluated, for purposes of apple production, by ascertaining the composition of a large number of varieties which were found to behave in high degree as one. In so far as these analyses permit comparison of varieties with themselves in different years, the results accord with the behavior of the group. The group swung together through a range of chemical composition, its place within the range in any year being determined by the conditions of the year. In so far as internal evidence indicates, the varieties here dealt with behave in the same manner as the group. It appears justifiable to consider that the cases in which only a single analysis was made were mani-

festing the same general behavior, and that an analysis made in 1920 or 1924 presents a close approximation to the minimum sugar content and maximum astringent content to be encountered in the variety under normal cultural conditions at Washington; that an analysis in 1923 or 1921 may be regarded as near the maximum sugar and minimum astringency to be expected here; and that 1922 will show an absence of extremes, the results standing near the middle of the range for both constituents.

The acid-astringency-sugar ratio, that is, the ratio obtained by dividing the total astringency and the total sugar content by the titratable acidity, has been calculated for each analysis and the results are included in Table 2. The importance of this ratio has been pointed out elsewhere (7); it attempts to derive from the analytical data an expression of the character of the material as judged by the sense of taste. Since sugar and astringent materials are influenced in opposite directions as regards their quantity by a given set of climatic conditions, it follows that the ratio will vary from year to year with varying climatic environment. The absolute amount of variation in any one constituent may not be great, but the collective effect of the simultaneously occurring variations in opposite directions of two constituents which are concerned in determining palatability or dessert quality is large. It is responsible for the wide differences in palatability or appeal to the taste which are observable in the fruit or juice of the same variety from season to season. The acid-astringency-sugar ratio is an attempt to sum up in one expression the factors responsible for the collective effect upon the taste organs of the constituents which they perceive. It furnishes a sort of shorthand summary of an analysis, permitting much more convenient and rapid comparisons between analyses than is otherwise possible. Thus a glance at the ratios in Table 2 shows at once that the juice of Bigg was much more acid and astringent in 1924 than in 1923; that Bloomfield and Connett Sweet were more astringent and less sweet in 1922 than in 1923, that the juices of Crookstem were very closely similar in 1922, 1923, and 1924, while those of Carlough were much more astringent in 1922 than in 1924. Such comparisons made directly from the analytical data demand several separate mental calculations with resulting lack of clarity. While the acid-astringency-sugar ratio tells nothing as to the absolute amounts of any of these constituents, it states their quantitative relationship to one another, and it is this quantitative relationship which is perceived by the sense of taste and expressed as a verdict of "too sweet," "too acid," "just right," or similar phrases. It is certain that many of the chemical analyses of apples and other fruits in the literature fall short of conveying as adequate conceptions of the organoleptic quality, the appeal to the taste, as they would convey had data upon the astringency content been included. The inclusion of such data in analyses, with the conversion of the analytical results into the form of the acid-astringency-sugar ratio, will materially aid the reader in deriving a conception of the effect upon the taste organs from the analytical data.

#### SUMMARY

Data upon the chemical composition of the juices of 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., in the years 1920 to 1924 are presented.

Two of the varieties were analyzed in 3 years, 63 in 2 years, and 33 in 1 year only. Analyses of 10 varieties of French cider apples grown at the Virginia Agricultural Experiment Station, Blacksburg, Va., 3 of which are also represented in the material grown at the Arlington farm, are also included in the tables. Many, perhaps most, of the 105 varieties concerned are not now represented by analyses in the literature.

The analytical results lend confirmation to the conclusion established by previous work that climatic conditions during the period of development and maturity produce consistent and sustained effects upon the chemical composition of the fruit of apple trees, the character of these effects being that of mass responses given by large groups of trees of dissimilar origin, adaptation to local conditions, and character of fruit.

Since the climatic conditions of the years in which the analyses were made are definitely known, and their specific effects upon the composition of their respective crops have been determined for large groups of varieties, any individual analysis has somewhat greater significance than would be the case were it not accompanied by such information.

#### LITERATURE CITED

- (1) ALWOOD, W. B.  
1903. ORCHARD STUDIES. IX. AN INVESTIGATION INTO THE CHARACTER OF CIDER APPLES IN EUROPE AND COMPARISONS WITH AMERICAN FRUIT. Va. Agr. Expt. Sta. Bul. 136, p. 71-96.
- (2) ——— and DAVIDSON, R. J.  
1904. ORCHARD STUDIES. XVI. THE COMPOSITION OF APPLES. Va. Agr. Expt. Sta. Bul. 143, p. 283-299.
- (3) ——— DAVIDSON, R. J., and MONCURE, W. A. P.  
1904. THE CHEMICAL COMPOSITION OF APPLES AND CIDER. U. S. Dept. Agr., Bur. Chem. Bul. 88, 46 p.
- (4) BIGELOW, W. D., GORE, H. C., and HOWARD, B. J.  
1905. STUDIES ON APPLES. I. STORAGE, RESPIRATION AND GROWTH. II. INSOLUBLE CARBOHYDRATES OR MARC. III. MICROSCOPIC AND MACROSCOPIC EXAMINATION OF APPLE STARCH. U. S. Dept. Agr., Bur. Chem. Bul. 94, 100 p., illus.
- (5) BROWNE, C. A., Jr.  
1900. A CHEMICAL STUDY OF THE APPLE AND ITS PRODUCTS. Pa. Dept. Agr. Bul. 58, 46 p.
- (6) CALDWELL, J. S.  
1925. SOME EFFECTS OF SEASONAL CONDITIONS UPON THE CHEMICAL COMPOSITION OF AMERICAN GRAPE JUICES. Jour. Agr. Research 30: 1133-1176, illus.
- (7) ———  
1928. CHEMICAL COMPOSITION OF APPLE JUICES AS AFFECTED BY CLIMATIC CONDITIONS. Jour. Agr. Research 36: 289-365.
- (8) ———  
1928. CHEMICAL COMPOSITION OF AMERICAN-GROWN FRENCH CIDER APPLES AND OTHER APPLES OF LIKE CHARACTER. Jour. Agr. Research 36: 391-406.
- (9) GORE, H. C.  
1908. UNFERMENTED APPLE JUICE. U. S. Dept. Agr. Bur. Chem. Bul. 118, 23 p., illus.
- (10) JONES, J. S., and COLVER, C. W.  
1912. THE COMPOSITION OF IRRIGATED AND NON-IRRIGATED FRUITS. Idaho Agr. Expt. Sta. Bul. 75, 53 p.
- (11) RICHARDS, E.  
1887. ANALYSES OF APPLES. U. S. Dept. Agr. Rpt. 1886: 350-355.
- (12) VAN SLYKE, L. L.  
1904. A STUDY OF THE CHEMISTRY OF HOME-MADE CIDER VINEGAR. N. Y. State Agr. Expt. Sta. Bul. 258, p. 439-494.





# BACTERIAL HALO SPOT OF KUDZU CAUSED BY *BACTERIUM PUERARIAE* HEDGES<sup>1</sup>

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## INTRODUCTION

The first appearance of the kudzu vine (*Pueraria thunbergiana*) in America is said to have been at the Philadelphia Centennial Exposition in 1876, where it was used to cover the Japanese tea houses. Once introduced it remained, these few plants having been left behind by the departing Japanese in the hands of a few fanciers of foreign plant oddities. In recent years, however, the vine has become of some economic importance in the South as a forage crop, a fact which has resulted in an increased interest in the problems connected with its culture and the diseases affecting it.

## HISTORY OF THE DISEASE

In August, 1925, badly spotted leaves of kudzu (pl. 1, A, B, C, and E), collected August 27 at Cairo, Ga., by O. C. Boyd, of the Georgia State Board of Entomology, were received by the writer with the statement that the "infection is just now becoming prominent." A month later a second lot of material was received, with the information that the disease had spread very little in the fields during the time that had elapsed since the submitting of the first specimens. It had been found in another field, however. No general survey had been made. The weather had been unusually hot and dry. The disease proved to be bacterial. The parasite was isolated, and laboratory and greenhouse work was carried on through the winter in Washington.

In June, 1926, a badly diseased vine found June 3 at Sylvester, Ga., was received from Boyd. There were few, if any, sound leaves on the plant, and this, wrote Boyd, was typical of all vines in a 5-acre field planted with roots obtained from Florida. The owner had rooted out all the vines in this field, hoping to prevent the spreading of the disease to an adjoining 30 acres of kudzu planted with Georgia roots. At this time the latter field was free from disease with the exception of a few rows adjacent to the field of diseased Florida kudzu. Later in the summer, however, Boyd found the whole of the 30-acre field of Georgia kudzu very badly infected, the disease being present in a most virulent form.

In the latter part of June, 1926, the writer visited kudzu fields in the vicinity of Cairo, Ga., and Monticello, Tallahassee, and Gainesville, Fla.

Much of the disease was present in the Cairo, Ga., field from which the original material had been obtained the preceding August.

<sup>1</sup> Received for publication Nov. 12, 1927; issued May, 1928.

Three fields were visited which had been planted with roots from this field, one in March, 1925, and two in February or March, 1926, i. e., one the spring preceding and two the spring following the discovery of the disease in August, 1925. Many young infections were found on leaves and runners in all three of these fields. In one of the 1926 plantings the young foliage of some of the vines was thickly beset with small angular water-soaked spots (pl. 1, D), from which the kudzu organism was later isolated.

Little, if any, of the disease was observed in the other five fields visited in the vicinity of Cairo.

The disease was also found in the vicinity of Tallahassee, Fla., though to a minor extent. It was not observed either at Monticello or Gainesville, Fla.

### GEOGRAPHICAL DISTRIBUTION

The writer has thus far observed this disease only in Georgia and Florida, but no extended survey has been made. In the summer of 1924 Clinton<sup>2</sup> reported in Connecticut a kudzu bean spot, of which he wrote: "New to State and probably to the United States; produced yellow spots on leaves exactly like wildfire of tobacco, but so far have failed to produce the disease; some spots are like the bacterial spot of bean." In 1926 dried specimens of this material were sent to the writer by Clinton for comparison. The disease appeared to be the same as that here described.

### DESCRIPTION OF THE DISEASE

The disease forms conspicuous spots on the leaves and glistening brown streaks on the succulent young runners. The infections are stomatal and the youngest spots are angular and water-soaked. (Pl. 1, D.) Later the center becomes brown and is surrounded by an irregular translucent area. (Pl. 1, A, C, E.) Slightly older spots have in addition a wide yellow halo. (Pl. 1, B.) Finally the surrounding tissue becomes brown before the diseased area has withered and dried. The bacteria are abundant in the center of the spot, but in the halo they are present chiefly if not exclusively in the bundles, from the cut ends of which in free-hand sections they pour out in masses.

The wide yellow halo, from which the common name of the disease is derived, is strikingly like that in tobacco wildfire. It is much more striking when the spots on a leaf are comparatively few. When they are close together the halo is less in evidence or disappears altogether.

### ISOLATION OF THE PARASITE

The parasite is easily isolated from fresh material or even from that which has been kept dry in the laboratory for three or four weeks. The only difficulty which the writer has thus far experienced in this regard was in isolating the organism during a long-continued period of excessive heat in Washington, when both diseased material (collected four weeks previously) and poured plates were

<sup>2</sup> CLINTON, G. P. KUDZU-BEAN. U. S. Dept. Agr., Bur. Plant Indus., Off. Plant Disease Survey, Plant Disease Rptr. Sup. 42:354. 1925. [Mimeographed.]

kept at the unusually high room temperature (about 34° C.). (See "Temperature relations," p. 427.)

The leaves will stand a treatment with HgCl<sub>2</sub> 1:1,000 for 30 to 45 seconds, but such sterilization is not necessary. Equally good results were obtained by careful washing, either rubbing between thumb and forefinger under the tap or dipping in alcohol and then passing through a series of two to five tubes of sterile tap water, allowing the pieces of tissue to remain a few minutes in each. The colonies appear in one to two days.

#### INOCULATION EXPERIMENTS ON KUDZU

Attempts to grow kudzu from seed in the greenhouse met with very little success during the winter of 1925-26. Most of the seeds failed to germinate, and those that did produced only very stunted plants, all but four of which soon died. These four survivors were kept for some time in the hope that they would begin to grow, and though they never produced more than a few leaves, they were finally inoculated, as they were the only kudzu plants available. They were sprayed in an inoculating cage in the greenhouse with a water suspension of a 2-day-old beef-agar culture of the kudzu organism. At the end of 24 hours, during which they were continually moist, the plants were removed from the inoculating cage. They were under observation for six weeks, but only one of the four ever showed any sign of infection. This plant had produced a few new leaves since inoculation. The other three had made no growth at all. From this one infected plant typical colonies of the kudzu organism were isolated. Parallel inoculations on Lima bean produced excellent infections, as hereinafter described.

During the summer of 1926 kudzu vines were very successfully grown from seed in the greenhouse, and on these excellent artificial infections were obtained in August. The following method was employed: A few drops from a thinly clouded 24-hour-old beef-broth culture were poured on both sides of young leaves, which were then rubbed lightly with a cotton plug and sprayed with a fine spray of sterile water. Some of the leaves were covered with paraffined bags, but no attempt was made to keep the other inoculated leaves moist for a number of hours. The plants were placed in the shade, however. The cultures used were from three different sources—the 100 per cent infected field in Sylvester, Ga., the Cairo field in which the disease was originally discovered, and a 1926 planting from the latter. All were from isolations made in June and July, 1926.

A week after inoculation there were excellent infections from all three strains, although the Sylvester organism seemed to be the most virulent. There were masses of very small, angular, water-soaked spots on the under surface of the leaf, in every way similar to those observed in the kudzu fields in June. On the upper surface the spots were brown. Some had large pale-green halos. The leaves in the paraffined bags were no more seriously infected than the others.

#### INOCULATION EXPERIMENTS ON LIMA BEAN

Owing to the difficulty experienced in growing kudzu satisfactorily in the greenhouse during the winter of 1925, all but one of the inoculation experiments carried on during that period were made on Lima

beans after a preliminary set of prick inoculations had shown that the kudzu organism was infectious to the Lima bean. Prick inoculations in stem and leaf blade resulted in a yellowing of the inoculated leaves, a reddening of the veins, and a subsequent wilting and shriveling. The organism was reisolated from such leaves.

Spray inoculations on Lima beans kept moist in inoculating cages for 24 hours after spraying with water suspensions of young beef-agar cultures, produced excellent infections in the greenhouse. In 8 to 10 days the leaves were covered with small irregular water-soaked spots. (Pl. 1, H, I.) By reflected light the whole spot appeared water-soaked. By transmitted light there was a pale-green halo with an inner red ring and a paler yellowish green or colorless center. Occasionally the center was red also. Four or five weeks after inoculation, the infected areas, much increased in size, were pale yellowish green or reddish brown, sometimes with a darker red border surrounded by a more or less yellowed area. Some of the veins in the infected areas were much reddened. In such an infected area covering about 8 sq. cm., bacteria were found in abundance five weeks after inoculation, and the kudzu organism was reisolated.

Excellent secondary infections appeared on pods (pl. 1, G) borne on Lima-bean plants inoculated by spraying when in blossom. Infections had been observed on the young leaves of these plants 11 days after inoculation. The spots on the pods were water-soaked and there was a white bacterial ooze. The older spots had reddish or yellowish centers and were considerably sunken. From both the white ooze and the water-soaked tissue the kudzu organism was reisolated.

Prick inoculations on green Lima-bean pods in a damp chamber produced water-soaked areas on both pods and seeds. (Pl. 1, F.) The first signs of infection were visible in two days. The small, tender, green seeds were especially susceptible.

The pathogenicity of the kudzu organism repeatedly isolated from artificial infections on Lima beans was proved by inoculation experiments.

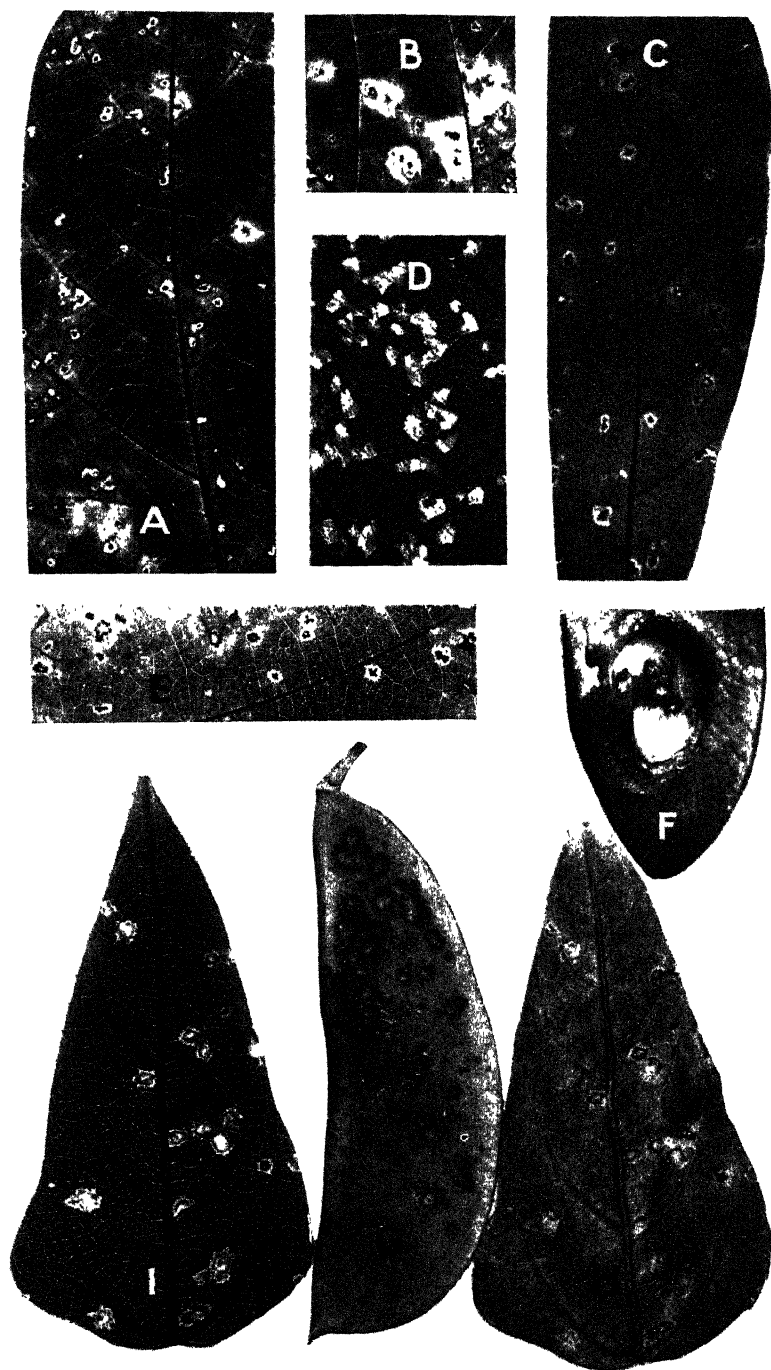
#### DISSEMINATION AND CONTROL OF THE DISEASE

No experiments in the dissemination and control of the disease have, as yet, been carried out, but there is some circumstantial evidence that the disease may be introduced into a new field by planting roots or runners from a field in which the disease is present (p. 420). The causative parasite, *Bacterium puerariae*,<sup>3</sup> is capable of long-continued growth at low temperatures (p. 427), and the writer believes that the organism is able to live over the winter in the dead leaves, bits of which may be left clinging to the transplanted roots and act as centers of infection in the spring. In practice no special attempt has been made to remove such debris before transferring the roots to a new field.

The planting of runners from an infected field is also a dangerous practice, as the succulent young stems are very susceptible and might

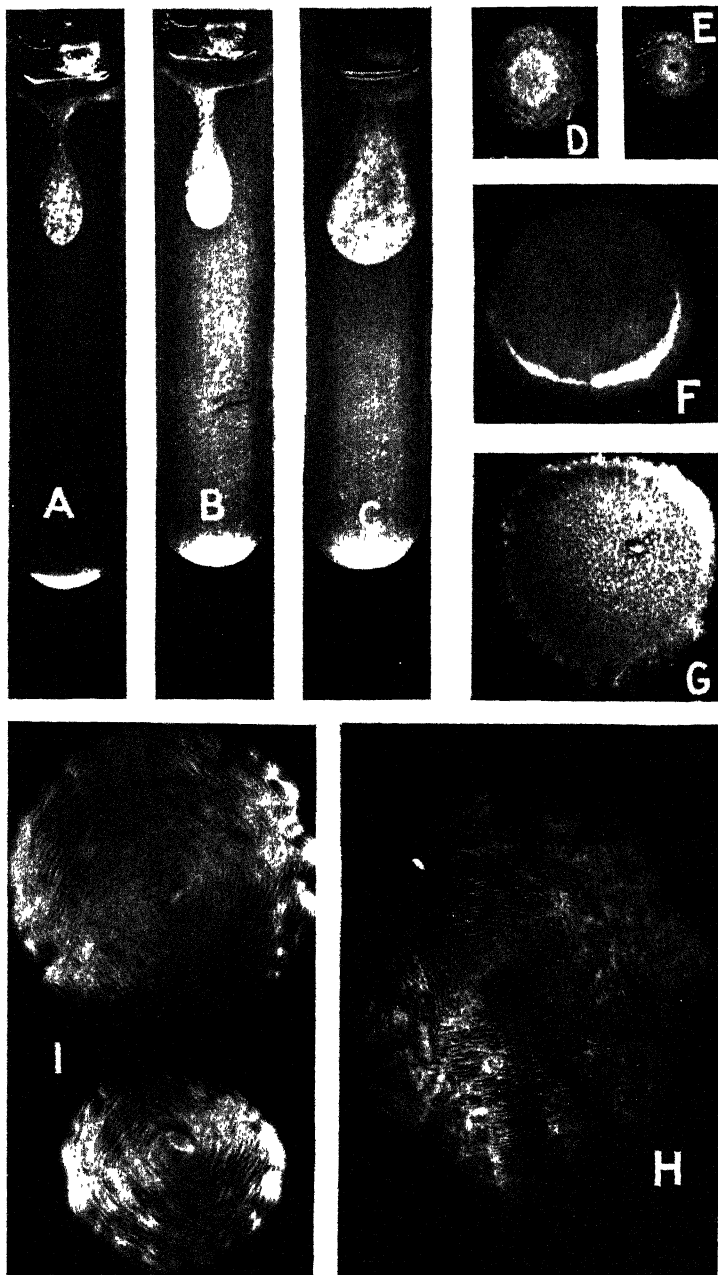
<sup>3</sup> An abstract of a preliminary paper presented before the American Phytopathological Society has appeared as follows:

HEDGES, F. BACTERIAL HALO SPOT OF KUDZU. (Abstract) Phytopathology 17: 48. 1927. (Through typographical error the name of the causal organism appears in this paper as *Bacterium pueriae* instead of *Bact. puerariae*.)



## BACTERIAL HALO SPOT

A-E.—Natural infections on kudzu: A-C and E, older spots showing halo,  $\times 1$ ; D, early water-soaked stage photographed by transmitted light,  $\times 5$ ; E, photographed by transmitted light. F-I.—Artificial infections on Lima bean: F, infected seed, photographed 9 days old; G, secondary infection on pod borne on plant inoculated by engraving when in blossom; H, same.



## CULTURES OF BACTERIUM PUERARIAE

- A.—Gelatin culture 5 days old. Saccate liquefaction. No liquefaction the fourth day.  $\times 1$   
 B.—Same, 24 hours later.  $\times 1$   
 C.—Same at end of 2 weeks.  $\times 1$   
 D, E.—Colonies on peptonized beef-infusion agar, 2 days old, showing white centers.  $\times 10$   
 F.—Same colony as D, 3 days old.  $\times 10$   
 G.—Colony growing next to the glass, 13 days old.  $\times 10$   
 H.—Colony 9 days old.  $\times 10$   
 I.—Colony 10 days old.  $\times 8$

serve as direct carriers of the disease. As the percentage of plants obtained from cuttings is very low, according to one of the oldest growers of this plant in America, there is a twofold reason for discontinuing the practice altogether.

In the present state of knowledge the writer can only recommend the use of well-established roots from which bits of dead leaves and runners have been removed. These should be selected from disease-free fields. As a further precaution the discontinuance of planting from runners is advisable.

#### CULTURAL CHARACTERS OF THE PARASITE

Cultural studies of *Bacterium puerariae* gave the results stated in the following paragraphs. All cultures were grown at room temperature unless otherwise stated.

**BEEF-INFUSION (PEPTONIZED) AGAR PLATES.**—Colonies appear in 1 to 2 days (21°–23° C.), and even when very young (2 to 3 days) show by oblique light the internal crosshatched markings or concentric striae which characterize them. (Pl. 2, D, E, H, I.) In occasional platings the young 2-day-old colonies have a very white center (pl. 2, D and E), which soon disappears, leaving a partial white ring, irregular in width, near the margin. (Pl. 2, F.) At first the colonies are round or approximately so, but later the margin becomes crenate. (Pl. 2, H and I.) This characteristic has been observed as early as the second day but is more commonly found in colonies a week or more old. When very much lobed the colonies become somewhat contoured. Colonies are bluish white, becoming somewhat opalescent, smooth, shining, and translucent. Young colonies are often very convex, older colonies almost flat; 3 to 6 mm. in diameter, sometimes in thinly sown plates, 9 to 10 mm. in diameter. No greening of the agar has ever been observed in plate cultures. Buried colonies are broadly fusiform, and those next to the glass are round and thin with coarsely granular centers and thinner regular or crenate margins. (Pl. 2, G.)

**BEEF-INFUSION (PEPTONIZED) AGAR SLANTS,  $P_H$  6.8–7.0.**—Thin whitish filiform growth in 24 hours (21°–23° C.), flat, glistening, finely contoured, translucent, butyrous, little if any odor. The growth shows a tendency to become smoother as the cultures grow older. Growth is always rather scanty or moderate, never copious. Slight but distinct greening of the medium is usually visible in about 48 hours.

**BEEF-INFUSION (PEPTONIZED) AGAR STABS,  $P_H$  7.0.**—Good whitish nailhead, scanty growth in upper part of stab. Slight but distinct green fluorescence usually noticeable in upper part of tube in about two days.

**BEAN-PLANT AGAR.**—Very scanty, barely visible growth in 24 hours. Thin, whitish, shining, somewhat contoured growth and slight green fluorescence in three days. The organism never made more than a scanty growth on this medium.

**NUTRIENT (BEEF-INFUSION) GELATIN,  $P_H$  7.2.**—Slow saccate liquefaction of gelatin (pl. 2, A–C), beginning in 5 to 13 days (temperature varied widely, 10°–22° C.). In some cultures the liquefaction finally became stratiform. Complete liquefaction has never been observed, although the cultures have been under observation for three and one-half to seven and one-half months. Green fluorescence was produced in 2 to 4 days.

**POTATO CYLINDERS (STEAMED).**—Potato considerably grayed in 24 hours (21°–23° C.). Growth barely visible at this time; usually a moderate growth in 2 to 6 days, cream colored, wet shining, and smooth when thick enough to hide the irregularities in the surface of the potato. Sometimes there is only a scanty growth.

**BEAN JUICE.**—Moderately clouded in 24 hours. Slightly green fluorescence in three days.

**BEEF-INFUSION (PEPTONIZED) BOUILLON,  $P_H$  7.0–7.2.**—Clouding in 24 hours. Faint rim and flocculent pellicle in older cultures; flocculence. Good growth in this medium, best near the surface. There is often a marked zoning, i. e., a heavy clouding in the uppermost part of the culture to the depth of about 1 cm., then a comparatively clear zone, and again good clouding in the lower half of the culture. Usually a slight blue-green fluorescence in about six days.

FERMI'S SOLUTION.—Clouding in 24 hours (23°–26° C.). Beautiful bluish green fluorescence in 6 to 14 days. A heavy pellicle is formed, which breaks up easily and is precipitated in flakes; wide white rim. An excellent medium for this organism.

LITMUS MILK.—No acid; alkali produced in three to five days. No coagulation; slow peptonization beginning in three to four weeks, not complete in two months. Litmus not reduced.

METHYLENE-BLUE MILK.—Only a trace of blue remaining in five days. Color slowly returned.

USCHINSKY'S SOLUTION.—Growth resembles that in Fermi's solution. Blue-green fluorescence, wide white rim, heavy pellicle breaking up easily into flakes.

### DIASTASIC ACTION

To test the diastasic action of *Bacterium puerariae*, streak inoculations were made on beef-extract agar plus 0.2 per cent cornstarch in Petri dishes. *Bact. phaseoli*, which has a strong diastasic action, was used as a check. The seventh day a saturated solution of iodine in 50 per cent alcohol was poured into the plates. There was some diastasic action with all the six colonies of *Bact. puerariae* tested, but only two of them produced a clear zone entirely free of starch. This zone varied from 1 to 5 mm. in width and was clearly visible to the naked eye. When the plates inoculated with the other four colonies were examined with the binocular microscope it was observed that much of the starch had been consumed, although enough was left to make the zone blue. The amount of diastasic action produced in a given culture varied along the different portions of the streak and, strangely enough, did not appear to be correlated with the amount of growth. In the *Bact. phaseoli* checks there was a perfectly clear zone 2 to 3 cm. wide on either side of the streak in which no starch at all was observed even with the binocular microscope.

### FERMENTATION OF SUGARS AND ALCOHOLS

#### ACID PRODUCTION

In the first series of fermentation experiments beef-extract agar (containing 0.5 per cent peptone,  $P_H$  6.6) was used as the basic medium. To this was added 1 per cent of the sugar to be tested and brom cresol purple as an indicator.

Acid was produced from all but one (lactose) of the five sugars tested and from one of the two alcohols, as follows:

GLUCOSE.—Acid produced in 24 hours; acid throughout in two to three weeks. Temperature, 19°–24.5° C.

SACCHAROSE.—Acid produced in two days. Temperature, 20.5°–23.5° C.

GALACTOSE.—Acid produced in two days. Temperature, 21°–23° C.

LEVULOSE.—Acid produced in four days. Temperature, 21°–23° C.

LACTOSE.—No acid produced. Under observation five weeks. Temperature, 21.5°–25.5° C.

GLYCERIN.—Acid produced in four days. Temperature, 21°–23° C.

MANNIT.—No acid produced. Under observation 16 days. Temperature, 21°–23° C.

Good growth on glucose, saccharose, galactose, levulose, and glycerin. Poor growth on lactose and mannit.

A second series of experiments was made, using ammonium phosphate agar (peptone free) as a base. This medium, originally  $P_H$  5.9, was adjusted to  $P_H$  7.0 before the addition of the sugars and alcohols. The results were similar to those obtained with the beef-



extract base. Another sugar (maltose) was tested, however. Brom cresol purple was the indicator, as in the preceding series. The results were as follows:

GLUCOSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.

SACCHAROSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.

GALACTOSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.

LEVULOSE.—Acid produced in three days. Temperature, 21°–23° C.

LACTOSE.—No acid produced. Temperature, 21°–23° C.

MALTOSE.—No acid produced. Temperature, 21°–23° C.

GLYCERIN.—Trace of acid in two days. Some of the cultures were acid throughout in three days. Temperature, 21°–23° C.

MANNIT.—No acid produced.

As in the first series of experiments, good growth was correlated with acid production. There was excellent growth on glucose, saccharose, galactose, levulose, and glycerin and barely distinguishable growth on lactose, maltose, and mannitol.

#### ALKALI PRODUCTION

Alkali production was studied on three sugars, using beef-extract agar (same stock as in the acid-production studies) with phenol red as a base, with the following results:

GLUCOSE.—Alkali produced in three or more days. Temperature, 19°–24.5° C.

SACCHAROSE.—Alkali slowly produced; none in two weeks, but alkaline throughout in seven weeks. Temperature, 20.5°–23.5° C.

LACTOSE.—Alkali produced. Temperature, 21.5°–25.5° C.

#### GAS PRODUCTION

No gas was produced from glucose, saccharose, lactose, or glycerin in ammonium phosphate agar slabs. Temperature, 22°–24° C.

#### NITRATE REDUCTION

The sulphanilic acid  $\alpha$ -naphthylamine test described in the manual of the Society of American Bacteriologists was used, with *Bacillus phytophthorus* as a check. Tests were made at the end of 24 and 48 hours and 4, 7, and 10 days. There was no reduction of potassium nitrate to nitrite by *Bacterium puerariae*. Ammonia produced; no gas. Temperature 23° C. *Bacillus phytophthorus* used as a check, reduced the nitrate in 24 hours.

#### PRODUCTION OF AMMONIA, HYDROGEN SULPHIDE, AND INDOL

Cultures 48 hours old of *Bacterium puerariae* in peptone beef-extract broth, with and without potassium nitrate, showed the presence of ammonia when tested with Nessler's solution.

No hydrogen sulphide was produced in peptone beef-infusion broth  $P_H$  7.0 and  $P_H$  7.4. Strips of filter paper saturated with lead acetate solution and suspended in the neck of the tube served as the indicator. *Bacillus coli* and *Bacterium phaseoli* were used as checks.

The Gnezda oxalic-acid test for indol gave negative results with cultures of *Bacterium puerariae* in Dunham's solution made with peptone containing tryptophane. *Bacillus coli* was used as a check.

## MINIMUM, OPTIMUM, AND MAXIMUM HYDROGEN-ION CONCENTRATION

The first series of studies to determine the minimum, optimum, and maximum  $P_H$  was made in beef-infusion broth with  $P_H$  reactions ranging in a series of short steps from 4.9 to 9.8. Growth occurred in 19 hours in  $P_H$  5.6 to 8.8 (best in  $P_H$  6.5). At the end of three days no additional tubes had clouded. In five days the  $P_H$  9.0 was clouded, having become more acid and thus within the range of the organism. The  $P_H$  5.0 never clouded (under observation 16 days).

A similar series was tested, substituting beef-extract broth for beef infusion. In this experiment the growth range of the organism was slightly extended. The  $P_H$  reaction ranged from 4.9 to 9.5. In 19 hours there was growth in  $P_H$  5.5 to 9.1. In 43 hours  $P_H$  9.4 was also clouded.

The optimum  $P_H$  in the beef-infusion broth is 6.5 to 7.0; in the beef-extract broth there seemed to be equally good growth at 5.5, 6.0, 6.4, and 6.8.

No green fluorescence was produced in beef-extract broth. In the beef-infusion broth most fluorescence was produced at  $P_H$  8.0.

## OXYGEN REQUIREMENTS

There was no evidence of anaerobic growth in shake cultures or stab cultures covered with 10 c. c. of melted agar. Good surface growth occurred in both types of cultures. Beef-extract agar was used.

## FLUORESCENCE

*Bacterium puerariae* should probably be classed as a green fluorescent organism, although the greening produced in beef-infusion media might easily be overlooked. In early stages of growth it may usually be observed in the uppermost centimeter of both beef-infusion agar stabs and beef-infusion broth at about  $P_H$  7.0 to 8.0. Later this small amount of greening becomes diffused and is not easily distinguishable. When first observed in the beef-infusion broth it is slightly bluish green. No fluorescence is noticeable in plate cultures. It is more marked in beef-infusion gelatin stabs than in beef-infusion agar. There is slight greening of bean juice and bean-plant agar.

No fluorescence has been observed in beef-extract broth or agar.

Fermi's and Uschinsky's solutions become beautifully blue-green fluorescent.

## LONGEVITY

The organism is known to live at least four weeks in leaves kept dry in the laboratory at moderate room temperatures (about 21°-22° C.). It has been plated from such material and found to be pathogenic.

The vitality of the organism is much affected by high temperatures (see "Temperature relations," p. 427), and it is probable that many of the bacteria die rather quickly in the drying tissues during hot weather.

Cultures kept at room temperature were alive at the end of six weeks on beef-infusion agar slants in Fermi's solution and sterilized bean juice.

It is the experience of the writer that stock cultures in Fermi's solution and beef-infusion agar can be kept alive one and one-fourth to one and one-half years in the ice box at about 19° C., and beef-infusion agar cultures have been known to survive two years and four months at that temperature.

#### TEMPERATURE RELATIONS

A series of cultures in beef-infusion broth P<sub>H</sub> 7.1 were tested at 10 different temperatures ranging from 2.5°–33° C.

In 19 hours growth had occurred from 13°–29° C. There was no growth at 2.5°, 7°, 8°, or 33°. The optimum growth in 24 hours was at 20°–23°. At the end of four days the best growth was at room temperature (average 22+° C.). Growth finally occurred at all the temperatures. All clouded at the lowest temperature (average 2.2+°) in 5 days (one tube in 43 hours, a second in 4 days).

Cultures were kept in the 2.2+° C. compartment all through the winter (November 22, 1926, to March 9, 1927), transfers being made from them from time to time to test their vitality. The temperature ranged from 1.5°–2.5° during this period. On March 9 they were still alive, though somewhat less vigorous, the transfers taking 2 days to cloud instead of 24 hours.

To recapitulate: The optimum temperature is 20°–23° C., the maximum temperature above 33°, the minimum below 2.5°, and long-continued growth is possible at about 2.5°.

The inhibiting effect of high temperatures was demonstrated by the inability of the writer to isolate *Bacterium puerariae* from infected leaves when the room temperature at which both the diseased material and the poured plates were kept averaged 34° C. It was first thought that the organism had been killed in the tissues by the high temperature that had prevailed throughout the four weeks following the collection of the halo spot, but this proved not to be the case, inasmuch as successful isolations were made from the same material when the plates were put into the ice box at a temperature of 15°–19° immediately after pouring and kept there for the duration of the experiment.

To test further, at this juncture, the comparative effect of high room temperatures and the lower ones of the ice box, two parallel sets of cultures were made in Fermi's solution. One was placed in the ice box, the other kept at room temperature. A fairly constant temperature of 18.5°–19° C. was maintained in the ice box. The room temperature was 31°–34.5°. The cultures grew slowly at both temperatures at first, but at the end of 10 days the difference between them was striking. Those at the high room temperatures were only very thinly clouded, while those at 19° were heavily clouded. Both were bluish green fluorescent.

#### THERMAL DEATH POINT

The behavior of *Bacterium puerariae* in thermal death-point experiments (10 minutes at 48°–65° C.) was very erratic, a given culture sometimes containing a few individuals with a thermal death point a number of degrees higher than that of the remainder. A transfer from one such culture resisted 63°, though others from the same tube were killed by 60° and 58° C. None resisted 65°. The thermal death point of the majority is still in doubt.

## MORPHOLOGY

On beef-infusion agar the organism consists of rods occurring singly or in pairs. Chains and filaments are also formed. The rods are polar flagellate, having 1 to 3 flagella at one or both ends. Capsules not observed. In 24-hour-old cultures on peptone beef-infusion agar the rods measured 0.86 to 1.67 by 0.3 to 0.48  $\mu$ .

## REACTION TO STAINS

*Bacterium puerariae* is easily stained with carbol fuchsin and the "aqueous-alcoholic" solutions of gentian violet and basic fuchsin. It is Gram negative and not acid fast. No capsules were demonstrated with Ribbert's capsule stain. The flagella stain readily with the Casares-Gil flagella stain.

## BRIEF DESCRIPTION OF BACTERIUM PUERARIAE HEDGES

A short polar-flagellate noncapulate rod, 0.86 to 1.67 by 0.3 to 0.48  $\mu$ ; chains present; endospores absent; Gram negative; not acid fast; producing "halo spot" on the kudzu vine (*Pueraria thunbergiana*), also pathogenic to Lima bean; strictly aerobic; liquefying gelatin; no reduction of nitrates to nitrites or gas production in nitrate media; ammonia produced; no production of hydrogen sulphide or indol; no chromogenesis on beef-extract bouillon or agar; slight but distinct blue-green fluorescence in beef-infusion (peptonized) bouillon and agar; marked greening of nutrient gelatin and beautiful blue-green fluorescence in Fermi's and Ushinsky's solutions; slight diastasic action; produces acid from glucose, saccharose, galactose, levulose, and glycerin, none from lactose, maltose, or mannit; no gas produced from glucose, saccharose, lactose, or glycerin; growth on peptonized beef-infusion agar in 24 hours moderate, whitish filiform, flat, glistening, finely contoured; colonies on peptonized beef-infusion agar bluish white or opalescent, round becoming crenate or lobed, smooth, shining, translucent, with internal crosshatchings or concentric striae; no acid or coagulation produced in milk; production of alkali and slow peptonization in milk; no reduction of litmus in litmus milk; steamed potato cylinders grayed, growth cream colored; optimum  $P_H$  is 6.5; growth range in peptonized beef infusion broth is  $P_H$  5.6 to 8.8 (19 hours); optimum temperature for growth about 22° C.; maximum temperature above 33°; minimum temperature below 2.5°; thermal death point, see page 427.

## SUMMARY

This paper describes a disease of kudzu vine. The disease is characterized by a conspicuous spotting of the leaves, the presence of wide yellow halos when the spots are not too close together, and glistening brown streaks on the succulent young runners.

Halo spot is known to occur in Georgia, Florida, and Connecticut.

From the spots an organism, *Bacterium puerariae* Hedges, has been isolated and its pathogenicity proved by pure-culture inoculations. Its biological characters are herein described.

*Bacterium puerariae* is also infectious to the Lima bean.

Examination of kudzu fields furnished very good circumstantial evidence that the disease is introduced into new areas by planting them with roots from diseased fields. The organism has been proved capable of long-continued growth at low temperatures, and the writer believes that it lives over the winter in the dead leaves, bits of which remain attached to the transplanted roots and act as centers of infection in the spring.

Roots for transplanting should be selected from disease-free fields. The use of cuttings should be avoided, as the succulent young runners are very susceptible to infection and might easily serve as direct carriers of the halo-spot disease.

# CHANGES PRODUCED IN APPLES BY THE USE OF CLEANING AND OIL-COATING PROCESSES<sup>1</sup>

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## INTRODUCTION

In the past two years it has become necessary in several of the Rocky Mountain and Pacific Coast States to employ cleaning methods for the purpose of removing excessive spray residue from apples after harvest. These methods are of two general types—dry brushing or wiping the fruit and washing it in chemical solutions. The latter method is the more effective and is coming into general use. The cleansing liquids employed are usually dilute solutions of either an acid or a base. Hydrochloric has been found to be the best acid at strengths varying from  $\frac{1}{2}$  to 3 per cent by weight of the acid, depending upon conditions. Of the basic solutions, sodium hydroxide or sodium carbonate are generally used in strengths similar to those given for hydrochloric acid. Disinfectants are sometimes added to the washing solutions. A discussion of these processes has been reported by Heald, Neller, and Overley,<sup>2</sup> and it suffices here to state that the hydrochloric-acid treatment is conducted in tanks with varying degrees of agitation, or under spray pipes from which the liquid, under moderate pressure, is directed upon the fruit. Alkaline solutions are similarly used, except that the liquid is generally heated to about 43° C. In both cases the fruit is washed in water before it is dried and packed. Fruit that has been treated with an alkali, such as sodium hydroxide, is given a light coating of a mineral oil-paraffin mixture to retard shriveling of the skin. The oil is generally applied by means of revolving brushes.

It is probable that these cleaning processes affect the physiological activities of the fruit, particularly when they are followed by the addition of a protective coating of oil. The purpose of the present paper is, by presenting a comparison of treated and untreated fruit, to show some of the effects produced by these treatments. The fruit used in the experiments was commercially harvested, cleaned, and treated in an irrigated section of central Washington.

## EXPERIMENTAL METHODS

Special attention was given to the effects of the treatments upon the respiratory rates of the fruit. These were determined by measuring the evolution of carbon dioxide from duplicate or triplicate samples of from six to eight apples each. The maximum variation

<sup>1</sup> Received for publication Dec. 27, 1927; issued May, 1928. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 142, College of Agriculture and Experiment Station, State College of Washington.

<sup>2</sup> HEALD, F. D., NELLER, J. R., OVERLEY, F. L., and DANA, H. J. ARSENICAL SPRAY RESIDUE AND ITS REMOVAL FROM APPLES. Wash. Agr. Expt. Sta. Bul. 213, 56 p., illus. 1927.

between duplicate determinations was 9.68, the minimum 1.71, and the average 5.25 per cent.

By the method used, air was drawn through soda-lime towers to remove  $\text{CO}_2$ . It was next bubbled through a half-inch column of 10 per cent  $\text{H}_2\text{SO}_4$ , and then into desiccators containing the weighed fruits. The air was drawn out from the bottoms of the containers and through a series of  $\text{CO}_2$  absorption towers containing 0.3 normal  $\text{NaOH}$ . Carbon dioxide was determined by precipitating the carbonate with  $\text{BaCl}_2$  and titrating the excess of  $\text{NaOH}$ .

The air currents were drawn through the units at a fairly rapid rate for four hours each day. The determinations were made at laboratory temperatures, these being recorded on a thermograph. Although these temperatures fluctuated to a considerable extent, they do not introduce a variable factor into the comparative data obtained, since all the determinations of any given comparison were obtained at the same time and at the same temperature.

During the period in which respiration determinations were being made, duplicate samples of the same lots of apples were held in the same room to determine the loss in weight and moisture. These were stored unwrapped in open-top paper bags.

#### EFFECT OF BRUSHING AND WASHING ON RESPIRATION RATES AND LOSS IN WEIGHT

The first experiments were made to determine the effect of dry brushing upon the respiratory rate of the fruit. A rotating spiral-brush type of machine was used on Winesap apples that had been in cellar storage for about three and a half months. The rate of carbon dioxide evolution during the first six days after brushing was somewhat increased by this treatment, as is shown in Table 1. Where the dry brushing was preceded by an acid-spray treatment the respiration rate was 22 per cent greater than that of the untreated fruit.

TABLE 1.—*Respiration rates and loss in weight of dry-brushed and of acid-washed dry-brushed Winesap apples taken from cellar storage; experiments begun January 10, 1927* \*

Treatment	Carbon dioxide per 100 gm. of fruit				Increase over untreated fruit	Loss in weight in 14 days
	Jan. 12	Jan. 14	Jan. 16	Total		
Dry brushed.....	Mgm. 4.75	Mgm. 4.79	Mgm. 6.20	Mgm. 15.74	Per cent 12.10	Per cent 5.04
Sprayed with hydrochloric acid and dry brushed.....	5.49	5.45	6.19	17.13	22.00	5.83
Untreated.....	4.51	4.31	5.22	14.04	-----	2.93

\* The data are the average of duplicate determinations.

Two months after the first group of experiments was begun some of the same lots of fruit were treated with 2 per cent solutions of hydrochloric acid and sodium hydroxide at  $25^\circ \text{C}$ . for 10 minutes with constant mild agitation but without brushing or wiping. The respiratory rate for the first six days after treatment was slightly less than that of the untreated fruit. (Table 2.)

TABLE 2.—*Respiration rates and loss in weight of Winesap apples taken from cellar storage and treated with sodium hydroxide or with hydrochloric acid; experiments begun March 11, 1926*<sup>a</sup>

Treatment	Carbon dioxide per 100 gm. of fruit				Decrease over untreated fruit	Loss in weight, Mar. 11 to 18
	Mar. 13	Mar. 15	Mar. 17	Total		
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>	<i>Per cent</i>	<i>Per cent</i>
2 per cent sodium hydroxide.....	10.55	7.02	6.60	24.17	2.18	4.12
2 per cent hydrochloric acid by weight.....	10.23	6.89	5.99	23.11	6.52	3.07
Untreated.....	10.82	7.21	6.68	24.71	-----	3.31

<sup>a</sup> The data are the average of duplicate determinations.

During the first seven days after treatment the loss in weight of fruit treated with hydrochloric acid was practically the same as that of the untreated fruit (Table 2), but there was a considerably increased loss where sodium hydroxide was used. In the previous work with fruit from this lot it may be noted (Table 1) that dry brushing caused weight to be lost at a much increased rate and that an additional treatment with an acid spray caused but little further loss. An acid treatment without brushing or wiping likewise failed to produce an appreciable effect on the rate of loss. (Table 2.) Robinson and Hartman<sup>3</sup> also found that an acid-bath treatment had little effect on loss of weight as compared with the loss from wiping or brushing, which apparently disturbs and removes some of the protective wax coating. Heald, Neller, and Overley have shown, however,<sup>4</sup> that a brushing treatment which causes a rapidly increased loss of weight at laboratory or room storage has practically no effect on loss of weight when the fruit is held in cold storage at 32° to 33° C.

#### EFFECT OF OILING ON RESPIRATION RATES, LOSS IN WEIGHT, AND MOISTURE CONTENT

A considerable portion of the northwestern apple crop is prepared for storage and marketing by being coated with a thin film of oil after it has been cleaned by dry brushing or with a solution containing sodium hydroxide. In the process followed in these experiments the oil used was a highly refined, highly viscous mineral grade and was mixed with paraffin wax. It was warmed sufficiently to dissolve the paraffin and was spread over the surface of the apples by means of revolving brushes.

On November 1 some newly harvested Winesap apples that had been dry brushed and oiled were put in cold storage, together with some of the same lot that had been dry brushed but not oiled. At the same time untreated fruit was also stored. Four months later samples were brought to the laboratory for respiration determinations. The results show that whereas the respiratory rates of the brushed and untreated fruits were practically the same, they were both over 40 per cent higher than that of the oiled fruit. (Table 3.) In 1924 Magness and Diehl reported<sup>5</sup> data on the respiration of Winesap

<sup>3</sup> ROBINSON, R. H., and HARTMAN, H. A PROGRESS REPORT ON THE REMOVAL OF SPRAY RESIDUE FROM APPLES AND PEARS. Oreg. Agr. Expt. Sta. Bul. 226, 46 p., illus. 1927.<sup>4</sup> HEALD, F. D., NELLER, J. R., OVERLEY, F. L., and DANA, H. J. Op. cit.<sup>5</sup> MAGNESS, J. R., and DIEHL, H. C. PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE. Jour. Agr. Research 27: 1-38, illus. 1924.

apples which showed that when they were coated with mineral oil and with paraffin the evolution of carbon dioxide was reduced by about 40 per cent. Their results were similar to those of Table 3 and were obtained at approximately the same temperature as the present writer used. Magness and Diehl also made a study of the  $\text{CO}_2\text{-O}_2$  respiratory ratio and found that there was little anaerobic respiration in apples thinly coated with oil and stored at a temperature of  $18^\circ\text{C}$ . or lower. At  $26^\circ$ , however, there was considerable anaerobic respiration, with a consequent development of poor flavor. It was pointed out that this tendency became more pronounced as the thickness of the oil coating was increased.

TABLE 3.—*Respiration rates, water content, and loss in weight of brushed and of oil-coated Winesap apples after four months in cold storage; experiments begun February 28, 1927*<sup>a</sup>

Treatment	Carbon dioxide per 100 grams of fruit				Increase over oiled fruit	Water content Mar. 22	Loss in weight Mar. 1-22
	Mar. 2	Mar. 4	Mar. 6	Total			
	Mgm.	Mgm.	Mgm.	Mgm.	Per cent	Per cent	Per cent
Brushed.....	11.61	9.60	7.00	28.21	44.8	84.23	4.83
Oil coated.....	7.52	6.42	5.54	19.48	-----	84.05	4.51
Untreated.....	11.10	8.50	7.68	27.28	40.1	84.00	5.29

<sup>a</sup> The data are the average of duplicate determinations.

Loss-in-weight determinations showed that the unoiled fruits of Table 3 lost weight but little faster than the oiled fruits. The moisture content of the brushed, oiled, and untreated fruits was about the same. Figure 1 shows the air temperature and relative humidity that prevailed during the progress of the above experiment.

On June 22, or after nearly eight months of cold storage, other samples from the same lots of Winesap apples were subjected to respiratory measurements. The unoiled apples continued to respire at a faster rate than the oiled, although to a lesser extent. The increases amounted to 16.8 and 18.3 per cent, respectively, for the brushed and untreated lots. (Table 4.)

TABLE 4.—*Respiration rates of brushed and of oil-coated Winesap apples after eight months in cold storage; experiments begun June 20, 1927*<sup>a</sup>

Treatment	Carbon dioxide per 100 grams of fruit			Increase over oiled fruit
	June 22	June 24	Total	
	Mgm.	Mgm.	Mgm.	Per cent
Brushed.....	12.00	12.30	24.30	16.8
Oil coated.....	11.24	9.56	20.80	-----
Untreated.....	12.24	12.37	24.61	18.3

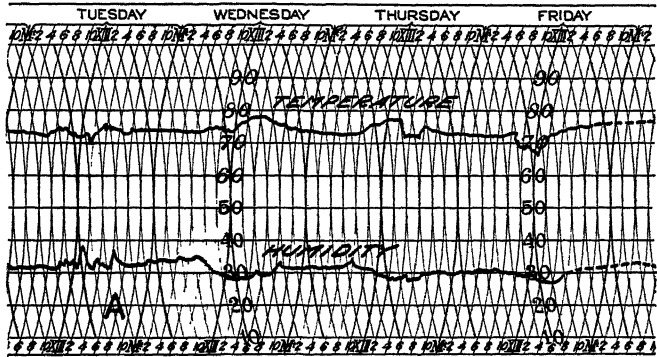
<sup>a</sup> The data are the average of duplicate determinations. Water content and loss in weight were not determined.

The process of brushing without oiling had little effect upon the respiration of these stored Winesap apples, as the brushed fruit respired slightly faster after four months and slightly slower after eight months of cold storage. (Tables 3 and 4.) Brushing had likewise little effect upon losses of moisture and of weight.



Samples of Delicious apples were prepared for storage in the same manner as were the Winesaps and they were kept in the same cold-storage room. As seen in Table 5, oil had a similar retarding effect upon respiration. This retardation amounted to 33.4 and 46.7 per cent, respectively, of the CO<sub>2</sub> evolution from the brushed and untreated lots. There was but little difference in the loss in weight of the different lots, but the moisture content of the oiled fruit was slightly higher.

MARCH 1, 1927



JUNE 21, 1927

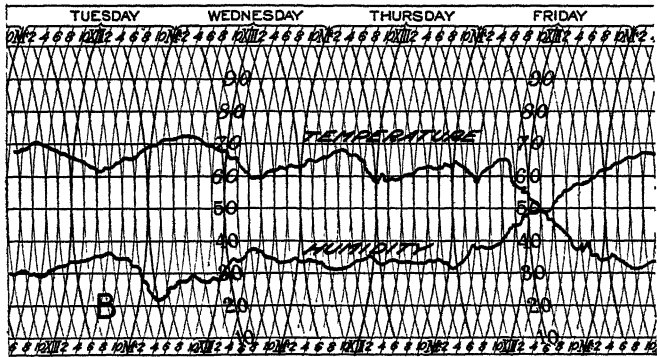


FIG. 1.—Temperature in degrees Fahrenheit and percentage of relative humidity that prevailed during respiration determinations of Winesap apples. A refers to the data in Table 3; B, to the data in Table 6

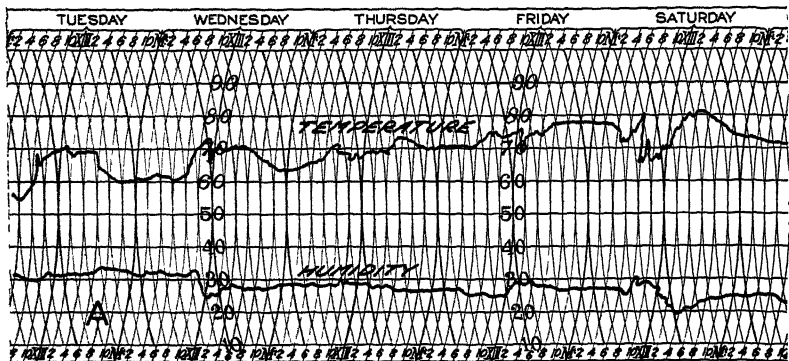
TABLE 5.—Respiration rates, moisture content, and loss in weight of brushed and of oil-coated Delicious apples after four months in cold storage; experiments begun February 21, 1927 <sup>a</sup>

Treatment	Carbon dioxide per 100 grams of fruit				Increase over oiled fruit	Loss in weight, Feb. 21–Mar. 15	Moisture content, Mar. 15
	Feb. 23	Feb. 25	Feb. 27	Total			
	Mgm.	Mgm.	Mgm.	Mgm.			
Brushed.....	15.1	19.2	17.2	51.5	33.4	5.04	83.59
Oil coated.....	12.2	14.4	12.0	38.6	-----	4.87	84.54
Untreated.....	17.0	20.1	19.5	56.6	46.7	4.75	83.77

<sup>a</sup> The data are the average of duplicate determinations.

The respiratory rates of the Delicious apples were again determined after a period of eight months in cold storage. At that time (Table 6) the untreated fruit respired 43.28 per cent more carbon dioxide than the oil-coated fruit. Figure 2 gives the air-temperature and relative humidity record for the time during which the respiration and loss of weight determinations with Delicious apples were in progress. Although the fluctuations in temperature and humidity were considerable, they were the same for the compared treatments as given in the above discussion.

FEBRUARY 22, 1927



JUNE 8, 1927

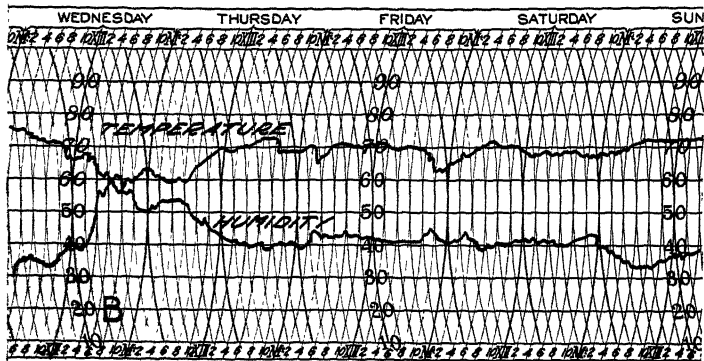


FIG. 2.—Temperature in degrees Fahrenheit and percentage of relative humidity that prevailed during respiration determinations of Delicious apples. A refers to the data in Table 5; B, to the data in Table 6

TABLE 6.—Respiration rates and loss in weight of oil-coated Delicious apples after eight months in cold storage; experiments begun June 7, 1927 \*

Treatment	Carbon dioxide per 100 grams of fruit			Increase over oiled fruit	Loss in weight, June 6-21
	June 9	June 12	Total		
Oil coated.....	Mgm. 9.95	Mgm. 12.46	Mgm. 22.41	Per cent	Per cent 4.73
Untreated.....	14.23	17.87	32.10	43.28	5.54

\* The data are the average of triplicate determinations. Water content not determined.

Dry brushing without oil coating slowed up the respiratory rate of Delicious apples as measured after four months of cold storage. (Table 5.) The data of Table 1 show a slight increase in the rate of respiration of Winesap apples as measured immediately after brushing. It is possible that the brushing process accelerated the respiration rate of the Delicious apples at first, resulting in less metabolic reserve when the respiration rates were determined four months later.

As shown in Tables 3 and 4, the effect of oiling was less apparent on the Winesap apples held eight months in cold storage than on those held four months. After eight months in storage the Winesap apples appeared very waxy, especially after warming up to room temperatures. This wax may have served to check respiration in much the same way as did the oil. The respiratory rate of the unoled Delicious apples after eight months continued to be as much in excess of the oiled fruit as it was after four months of cold storage. The Delicious apples, of course, did not become nearly so waxy as the Winesap apples.

#### EFFECT OF OILING ON QUALITY OF FRUIT

It has been shown that coating apples with a film of oil causes a marked reduction in the respiratory rate of the fruit even after eight months of cold storage. The practice of oiling is employed when the fruit has been brushed or cleaned in an alkaline solution, the object being to give the fruit an oil-film protection equal or superior to the natural waxy protective coating of the untreated fruit. Since the experiments show that this cleaning and oil-coating process causes the respiration rate of the fruit to be considerably lowered, the question arises as to the effect of this practice upon the keeping and dessert quality of the fruit.

A comparison of apples by tasting or eating did not reveal any significant difference in dessert quality between treated and untreated fruit kept in storage for different lengths of time. When waxy apples like the Winesap variety, are brought to room temperature after a long period in cold storage, the excessive development of wax often makes it difficult to differentiate between the oiled and unoled fruit even by visual examination. In most cases, however, the oil-coated fruit is easily recognized by its decreased luster and increased waxiness.

The oil-coated Delicious fruits could easily be distinguished from the unoled, and the flavor was also slightly different. This change in flavor could not be declared either beneficial or detrimental.

#### DISCUSSION

In general, the experiments here reported have shown that the oil-coated fruit loses weight and shrinks somewhat less rapidly than the unoled when brought into room or marketing temperature after long periods of cold storage. Since the oil-coated fruit also has a greater reserve of metabolic activity, as shown by respiration measurements, it is quite apparent that the practice of oil coating has a tendency to prolong the keeping quality of the fruit. In the fruit under test the dessert quality was neither noticeably benefited nor harmed. This fact indicates that the coating of oil as applied in these experiments was not sufficiently heavy to induce anaerobic respiration with its accompanying unpleasant flavors.

## SUMMARY

Winesap and Delicious apples grown in an irrigated section of eastern Washington were placed in cold storage immediately after harvesting and cleaning. The cleaning methods used were (1) dry brushing, (2) dry brushing and oil coating, and (3) mild agitation in dilute hydrochloric acid without brushing or wiping. When the fruit to be tested had remained in cold storage for a certain time the rates of respiration and losses of moisture and of weight were determined at room or marketing temperature.

After four months in cold storage untreated Winesap apples respired  $\text{CO}_2$  at a rate over 40 per cent faster than dry-brushed, oil-coated fruit. After eight months in cold storage the uncoiled Winesap apples respired about 17 per cent faster.

Untreated Delicious apples respired about 46 per cent faster than the brushed, oil-coated lot after four months, and about 43 per cent faster after eight months in cold storage.

When exposed to room or marketing temperatures of about  $20^\circ \text{C}$ . and relative humidities of about 30 per cent of the maximum, the uncoiled Delicious and Winesap apples lost weight at a moderately faster rate than the oil-coated fruit. The moisture content of the untreated was only slightly lower than that of the oil-treated apples.

A film of oil of the character and thickness used in the process employed in these experiments caused no significant difference in the dessert quality of the fruit after as much as eight months of cold storage. The oil coating tended to reduce the rate of shriveling and softening when the fruit was exposed to room or marketing temperatures.

Dipping in 2 per cent by weight of hydrochloric acid at  $20^\circ \text{C}$ . with mild agitation for 10 minutes had little effect upon the subsequent rates of either  $\text{CO}_2$  respiration or loss in weight of Winesap apples. Dipping in 2 per cent sodium hydroxide under the same conditions affected respiration slightly and increased the rate of loss in weight.

The use of hydrochloric acid under the above conditions, except that contact was made with the fruit by means of jet sprays, resulted in unchanged rates of respiration and in no change in rate of loss in weight.

Brushing the fruit in either the dry or the wet cleaning process caused the respiration rate to be greater, and markedly increased the rate of loss in weight when the fruit was exposed to marketing temperatures immediately after the brushing treatments. When the fruit was held in cold storage for several weeks, beginning immediately after a brushing treatment, this effect of brushing became less marked.

# CORRELATED STUDIES IN OATS OF THE INHERITANCE OF REACTION TO STEM RUST AND SMUTS AND OF OTHER DIFFERENTIAL CHARACTERS<sup>1</sup>

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## INTRODUCTION

Stem rust caused by *Puccinia graminis avenae* Erikss. and Henn., and the smuts, *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. & S.) Magn., are among the most destructive parasites of oats. Certain varieties of oats are resistant commonly to stem rust and others are immune from or resistant to the smuts. Stakman and others (14)<sup>2</sup> with stem rust and Reed (12) with the smuts have observed physiologic specialization. This complicates the problem of breeding resistant varieties, since it is difficult to control the physiologic forms of the disease under field conditions.

The stem-rust resistant parents used in these studies were segregates from crosses of White Russian with Minota or Victory. The White Russian variety has proved highly resistant to stem rust in the United States, and the rust-resistant segregates used as parents have been highly resistant at University Farm, St. Paul, Minn., for several years. They are resistant, therefore, to any or all forms of oat stem rust so far present in this locality. Black Mesdag was found by Reed (11) to be immune from attacks of both loose and covered smuts of oats, while Reed and Stanton (13) believed that resistance to both forms was dependent upon the same genetic factors. In the studies here reported no attempt has been made to use single physiologic forms of stem rust, and both loose and covered smuts have been present.

The purpose of the present paper is to present a study of the inheritance of reaction to stem rust and smuts in relation to other differential characters of the parents, more especially black versus nonblack glumes, presence of many or few hairs on the rachilla which supports the second kernel of the spikelet, and differences in awn development.

## PREVIOUS STUDIES OF INHERITANCE

Stakman, Levine, and Bailey (14) have demonstrated instances of physiological specialization for stem rust of oats, *Puccinia graminis avenae* E. and H. Garber (5) and Griffie (6) obtained a segregation in F<sub>2</sub> of 3 resistant, 1 susceptible, using stem-rust inoculum collected in Minnesota. Later generations could be explained also on a single-factor pair basis. In these studies White Russian was used as the resistant parent.

<sup>1</sup> Received for publication Dec. 29, 1927; issued May, 1928. Published with the approval of the director as paper No. 743, Journal series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 457.

Reed (12) observed physiological specialization in the smuts, although certain varieties appeared resistant to all races of smuts which he used. Black Mesdag proved immune from both covered smut, *Ustilago levis*, and loose smut, *U. avenae*.

Studies of reaction to smut in oats have demonstrated that immunity, resistance, and susceptibility are inherited characters. The difficulty of obtaining a heavy epidemic has made it impossible to determine the number and nature of the genetic factors involved without the use of more detailed studies than have been made so far. Wakabayashi (15) studied crosses of Red Rustproof, which is immune from covered smut, with Black Tartarian, which is susceptible. Immunity was dominant and several genetic factors were believed to be involved. Gaines (4) obtained similar results. Barney (1) suggested that reaction to loose smut in three different crosses could be explained upon a monohybrid, dihybrid, and trihybrid basis, respectively. The data presented, however, were not sufficient to prove the hypothesis. Reed and Stanton (13) in crosses of Fulghum, which is resistant to both loose and covered smut, with Swedish Select, which is susceptible, presented evidence to indicate that resistance to both forms was dependent upon the same genetic factors. Apparently both resistance and immunity are dominant over susceptibility. No cases of close linkage of the genetic factors which condition reaction to either stem rust or smuts and other differential characters have been reported.

Differences in awn development have been studied by numerous workers. Fraser (3) explained results which he obtained in crosses of Sixty Day and Burt on the basis of a single-factor pair for awning. His results agreed with those of Nilsson-Ehle (10) and Love and Craig (9), which indicate that the gene for yellow color of the glume inhibits awn development. Coffman, Parker, and Quisenberry (2) have reviewed most of the literature regarding the inheritance of weak and strong awns. In general, weak awns have proved dominant to strong awns and most of the results have been explained on a single main pair of genetic factors. Minor modifying factors are involved in some crosses, and environmental conditions may greatly modify the production of awns.

Black glumes have proved dominant or epistatic to other colors (8). In most cases black versus white have proved to be dependent upon a single-factor pair.

#### MATERIALS AND METHODS

The cultures used in these studies consisted of several apparently homozygous selections from crosses of Minota × White Russian or Victory × White Russian; Black Mesdag and  $F_1$  to  $F_5$  crosses between Black Mesdag and homozygous rust-resistant segregates from the White Russian crosses. Two of three strains used in the crosses with Black Mesdag have not been named. These are Minota × White Russian, Nursery Series Nos. II-18-37 and II-18-4. The Victory × White Russian selection, II-18-2, which was crossed with Black Mesdag, has been named "Anthony" and has been approved for distribution as the most promising stem-rust-resistant variety obtained so far. These three rust-resistant selections were obtained from the lines studied for reaction to rust by Garber (5) and by Griffiee (6).

The crosses between selections II-18-2 and II-18-4 and Black Mesdag were studied primarily only for glume color and rust reaction, although a considerable number of lines with white glumes were studied for smut reaction as well.

The most intensive study of inheritance was made with the cross between the selection II-18-37 of the White Russian  $\times$  Minota cross, and Black Mesdag. This selection produced open panicles, white glumes, none to a few weakly developed awns, and none to a few hairs on the pedicel or rachilla which is attached to the first or primary kernel of the spikelet and which supports the second kernel of the spikelet.

The Black Mesdag line was obtained from an oat classification nursery, the seed for which was obtained from Etheridge about 1920. At this time it was a pure-line selection. It has not been pedigreed recently and, except as mentioned later, appears homozygous. In contrast to the White Russian-Minota selection, it is susceptible to stem rust, immune from smut, produces black glumes, many strongly developed, geniculate awns, and many hairs on the rachilla of the second kernel of the spikelet. One plant of the 20 which were studied in 1923 deviated somewhat from the others. It was lacking in hairs on the rachilla, and the awns were more weakly developed than those of the other plants. It could have been obtained from a natural cross, and occasional natural crosses do occur in oats at University Farm, St. Paul (7). Such natural crosses are not frequent enough to seriously modify the ratios obtained in genetic experiments. They could explain an occasional aberrant type.

The studies of rust reaction were made by spraying the  $F_2$  field cultures in 1923 with spore suspensions of stem rust, *Puccinia graminis avenae*, collected at University Farm, St. Paul, and cultured in the greenhouse. An unusually heavy infection was obtained in 1923 and separations into resistant and susceptible plants were made easily. Resistance is completely dominant over susceptibility. Resistant plants were tested further by growing cultures in the greenhouse from the plants which were resistant in the field and inoculating approximately 30 seedlings of each with cultures of stem rust.

Studies on reaction to smut were made by using smut material collected at University Farm, St. Paul. No attempt was made to control the type of smut used and both *Ustilago avenae* and *U. levis* undoubtedly were present. An examination of the material used as inoculum was made in 1925 and 1926. The percentage of smooth spores was approximately the same as that of echinulated spores, indicating that nearly equal amounts of covered and loose smut were used. While it would have been more desirable in certain ways to have made separate studies of these two smuts, the Black Mesdag parent was immune from both, and as a rule most oat varieties have reacted in a similar manner to both smuts (11, 12). Reed and Stanton (13) in crosses of Fulghum with Swedish Select studied separately the reaction of  $F_2$  to  $F_3$  families to *U. avenae* and *U. levis* and concluded that the same genetic factors were responsible for reaction to both forms of smut.

Studies of reaction to smut are somewhat difficult as complete infection is seldom if ever obtained, and if it were obtained other

characters could not be studied at the same time. It seemed most important in these investigations to determine the proportionate number of immune lines without carefully studying the differences in degree of susceptibility in the susceptible and resistant lines. Individual  $F_2$  plants were studied for rust reaction, pubescence on rachilla, and awn development. Random selections of approximately equal numbers of  $F_2$  plants were selected in each of the four groups—black glumes resistant to rust, black susceptible, white resistant, white susceptible. Each such  $F_2$  plant became the parent of an  $F_3$  line. Twelve-foot rows were grown in  $F_3$  and sown at the rate of 75 seeds per row. The seed was smutted by mixing an even teaspoonful of smut spores with the grain in each envelope. The glumes were not removed from the kernels, as it seemed more desirable to use large numbers and test the immune lines for several years than to obtain more refined data on the susceptible lines. Lines which were susceptible in  $F_3$  were not tested again. All immune lines in  $F_3$  were again tested in  $F_4$ . Such lines were harvested in bulk in  $F_3$ , and 2 gm. of seed mixed with smut spores were used for each  $F_4$  row. All immune lines in  $F_4$  were again studied in  $F_5$ .

The  $F_1$  plants from which the  $F_2$  material originated were grown near Washington, D. C., in a greenhouse of the United States Department of Agriculture, and only the threshed grains were available; consequently, no data were taken on this  $F_1$ . The Washington greenhouse material was grown and harvested under the direction of T. R. Stanton, agronomist in charge of oat investigations of the Office of Cereal Crops and Diseases. Stanton's cooperation made possible the saving of a crop season by growing the  $F_1$  the same year that the cross was made. Several  $F_1$  plants were grown at University Farm, St. Paul, the same season as the  $F_2$ . The  $F_1$  and  $F_2$  were practically free from smuts.

Correlated studies of reaction to rust and smut and color of glume, awn development, and pubescence on rachilla of the second kernel of the spikelet were made by correlating the differential characters of the  $F_2$  plants with the  $F_2$  and  $F_3$  reaction to rust and the classification of smut reaction based upon the  $F_3$  to  $F_5$  data.

#### INHERITANCE OF DIFFERENTIAL CHARACTERS

The mode of inheritance of the differential characters, black versus white grain color, stem-rust resistance versus susceptibility, smut reaction weak versus strong awns, and few versus many hairs on the rachilla will be discussed, and following this a study of linkage or correlation of the characters will be made.

#### COLOR OF GLUMES

The  $F_1$  glumes were brownish black, a little lighter in color than the black parent and shading over to a light brownish color, depending apparently on the degree of maturity. There is some variation in a homozygous black variety, however, and no reliable differentiation into homozygous and heterozygous black glumed plants seems to be possible.

The results obtained in the first group of experiments are given in Table 1. The color of kernel was found to be dependent on a single-factor difference in these crosses, the results approximating a 3 : 1 ratio.



TABLE 1.—*Number of black and white glumed plants in F<sub>2</sub> from crosses between Black Mesdag and three white-glumed strains*

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Black-glumed plants	White-glumed plants	Black-glumed plants	White-glumed plants				
(White Russian×Victory) II-18-2× Black Mesdag.....	635	199	625.5	208.5	834	9.5	±8.43	1.13
(Minota×White Russian) II-18-37× Black Mesdag.....	2,907	1,028	2,951.2	983.8	3,935	44.2	±18.33	2.41
(Minota×White Russian) II-18-4× Black Mesdag.....	1,069	352	1,065.7	355.3	1,421	3.3	±10.98	.30
Total.....	4,611	1,579	4,642.5	1,547.5	6,190	31.5	±22.98	1.37

The greatest deviation of observed from expected numbers in a single F<sub>2</sub> group of crosses is 2.41 times the probable error, and for all F<sub>2</sub> plants the deviation is 1.37 times the probable error.

There were found occasionally a few small and unripe plants and their color was somewhat doubtful. This may have caused slight errors in the classification, but as a whole the two characters pigmented and nonpigmented glumes are easily distinguished and the results are in good agreement with expectation.

#### RESISTANCE TO STEM RUST

The resistance of the hybrid strains used as parents in the crosses here concerned originated from White Russian by the selection of homozygous resistant plants from crosses of a strain of this variety with Victory or Minota.

It appeared probable that also in the present crosses resistance might prove to be dominant and dependent on a single differential factor, as has been found in previous studies in which White Russian was one of the parents (5, 6). But there was, of course, a possibility that Black Mesdag differed from the varieties Victory and Minota in one or more factors, fundamental for development of resistance.

The data obtained from the F<sub>2</sub> generation of crosses between Black Mesdag and the resistant strains mentioned are given in Table 2. Results are quite similar for the three crosses, and the segregation approximates a 3:1 or monohybrid ratio. The greatest deviation was 1.52 times the probable error for a single group and 1.15 times the probable error for the total number of individuals.

TABLE 2.—*Number of resistant and susceptible plants in F<sub>2</sub> from crosses between Black Mesdag and three white-glumed rust-resistant strains*

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Resistant plants	Susceptible plants	Resistant plants	Susceptible plants				
(White Russian×Victory) II-18-2× Black Mesdag.....	625	209	625.5	208.5	834	0.5	8.43	0.06
(Minota×White Russian) II-18-37× Black Mesdag.....	2,942	993	2,951.3	983.7	3,935	9.3	18.33	.51
Minota×White Russian) II-18-4× Black Mesdag.....	1,049	372	1,065.7	355.3	1,421	16.7	10.98	1.52
Total.....	4,616	1,574	4,642.5	1,547.5	6,190	26.5	22.98	1.15

Practically all the white resistant plants were tested in the greenhouse during the fall of 1923 for rust reaction. From each  $F_2$  plant which was tested, 20 to 25 seeds were planted in 4-inch pots. The seedlings were inoculated by spraying the plants with a fine spray of water and brushing the seedlings lightly with rusted plants of the susceptible variety Victory (6).

The rust used was transferred to the susceptible seedlings in the greenhouse from rusted plants grown in the oat nursery. The chief aim of the greenhouse work was to determine which  $F_2$  plants were homozygous for rust resistance, and therefore no very accurate data on number of resistant and susceptible seedlings were taken. The plants were counted in an early stage before the rust was very well developed. All pots which showed a few susceptible seedlings were thrown away immediately after the data were taken, while the pots having only resistant seedlings were kept for a longer time. As a rule, however, the plants could be classified fairly accurately about eight days after inoculation. The results from 705 segregating  $F_2$  families were 10,575 resistant:3,378 susceptible plants. The deviation from a 3:1 ratio on the basis of numbers was 110, and the probable error was 34.5 individuals. Deviation P. E.=3.188. When the above-mentioned facts are taken into consideration, the results are in fairly good agreement with expectation, and they give additional evidence in support of the theory of a single-factor difference between the resistant and susceptible parents. On the same factor basis, a 2:1 ratio of heterozygous resistant to homozygous resistant  $F_2$  plants is expected. The actual results obtained are given in Table 3. The deviation of obtained from expected results is 1.9 times the probable error. The greatest deviation was in the first group of crosses (White Russian $\times$ Victory) II-18-2 $\times$ Black Mesdag, but in this case the deviation is only 2.24 times the probable error. A deviation as great as this is expected to occur on the basis of random sample once in every six or seven trials.

TABLE 3.—Number of homozygous and heterozygous  $F_2$  plants in the group white resistant from crosses between Black Mesdag and three white rust-resistant strains

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Homozygous plants	Heterozygous plants	Homozygous plants	Heterozygous plants				
(White Russian $\times$ Victory) II-18-2 $\times$ Black Mesdag	53	81	44.7	89.3	134	8.3	3.7	2.24
(Minota $\times$ White Russian) II-18-37 $\times$ Black Mesdag	238	468	235.3	470.7	706	2.7	8.5	.32
(Minota $\times$ White Russian) II-18-4 $\times$ Black Mesdag	91	156	82.3	164.7	247	8.7	5.0	1.74
Total	382	705	362.3	724.7	1,087	19.7	10.5	1.88

The reliability of the classification for rust resistance is of interest. There was quite a heavy rust epidemic in the oat nursery, which undoubtedly made it comparatively easy to classify the plants. Of about 1,090 plants, classified as white-seeded rust-resistant, which were tested in the  $F_3$  generation, there were 5 plants which, by later

test, were found to be homozygous for susceptibility. As these 5 constitute less than 0.5 per cent of the total number tested, it will be seen that the character rust resistance can be considered just as dependable as any other plant character. About 120 white-seeded susceptible  $F_2$  plants were tested in the greenhouse. Of these, 3 were without much doubt classified wrongly, as they segregated for rust resistance.

#### NUMBER OF HAIRS ON RACHILLA

For each parent, as well as each hybrid plant, six spikelets were used in the determination of hairiness. Two spikelets from the lower middle and upper part of the main panicle of each plant were studied. The hairs were counted under a binocular. Counts could be made accurately when a few or a medium number of hairs were present, but when the number was as high as 20 accurate counts were difficult. The average number of hairs on the rachilla which supports the second kernel of the spikelet was determined by obtaining an average for the six counts made from the panicle selected to represent each individual plant.

The results obtained are for random selections of  $F_2$  parent plants within the four groups, black glumes resistant to stem rust, black susceptible, white resistant, and white susceptible. The parents,  $F_1$  and  $F_2$  generations were grown in 1923. The data obtained are given in Table 4.

TABLE 4.—Average number of hairs on rachilla in Black Mesdag, rust-resistant selections, and  $F_1$  and  $F_2$  crosses of Black Mesdag with three rust-resistant selections

Parents or crosses	Number of plants in groups based on number of hairs on rachilla				
	0-7	8-15	16-23	24-31	32-39
Black Mesdag.....	1	3	14	2	-----
White Russian×Victory II-18-2.....	20	-----	-----	-----	-----
II-18-2×Black Mesdag $F_1$ .....	11	1	-----	-----	-----
Black rust resistant $F_2$ .....	208	33	26	2	1
Black rust susceptible $F_2$ .....	116	29	9	6	-----
White resistant $F_2$ .....	116	21	9	1	-----
White susceptible $F_2$ .....	43	4	2	-----	-----
White Russian×Minota II-18-37.....	30	-----	-----	-----	-----
II-18-37×Black Mesdag $F_1$ .....	15	-----	-----	-----	-----
Black resistant $F_2$ .....	132	21	6	1	-----
White susceptible $F_2$ .....	115	19	4	-----	1
White resistant $F_2$ .....	139	16	1	1	-----
White susceptible $F_2$ .....	128	9	6	1	-----
White Russian×Minota II-18-4.....	25	-----	-----	-----	-----
II-18-4×Black Mesdag $F_1$ .....	4	2	-----	-----	-----
Black resistant $F_2$ .....	87	22	23	3	2
Black susceptible $F_2$ .....	99	35	16	3	-----
White resistant $F_2$ .....	96	31	14	6	-----
White susceptible $F_2$ .....	71	22	3	-----	-----

The segregation for color of glumes and rust reaction was on a single-factor basis in both cases. To obtain an accurate idea of the segregation for hairs on the rachilla, it was necessary to determine whether there was a linkage between rust reaction or glume color and numbers of hairs on rachilla. In the blacks, the ratio of hair group 0-7 to the total numbers in the other groups was 2.5 : 1,

while in the whites the ratio was 2 to 1. In the resistant and susceptible groups similar ratios for numbers of individuals in group 0-7 to the numbers in the remainder of the groups was 2.4 and 2.3 : 1, respectively.

The tendency of a correlation between high number of hairs on rachilla and black glume color makes a correction necessary before studying segregation for hairs on rachilla. Within each cross the data for the white segregates were calculated on the basis of a total of one-third as many white-glumed segregates as black. The segregation for hairs on the rachilla on this basis is presented in Table 5.

TABLE 5.—Segregation for hairs on rachilla on the basis of one-third as many white as black glumed segregates

Cross	Color of glume	Number of plants in groups based on number of hairs on rachilla					
		0-7	8-15	16-23	24-31	32-39	Total
II-18-2×Black Mesdag F <sub>2</sub>	Black	324	62	35	8	1	430
II-18-2×Black Mesdag F <sub>2</sub>	White	116	18	8	1		143
II-18-2×Black Mesdag F <sub>2</sub>	Black and white	440	80	43	9	1	573
II-18-37×Black Mesdag F <sub>2</sub>	Black	247	40	10	1	1	299
II-18-37×Black Mesdag F <sub>2</sub>	White	89	8	2	1		100
II-18-37×Black Mesdag F <sub>2</sub>	Black and white	336	48	12	2	1	399
II-18-4×Black Mesdag F <sub>2</sub>	Black	186	57	39	6	2	290
II-18-4×Black Mesdag F <sub>2</sub>	White	67	21	7	2		97
II-18-4×Black Mesdag F <sub>2</sub>	Black and white	253	78	46	8	2	387

It is apparent from Table 4 that there is a dominance of few hairs over many. In the F<sub>1</sub> of cross of II-18-2×Black Mesdag 1 plant out of 12 was in group 8-15. Accordingly, on a single-factor pair basis, one-twelfth of the F<sub>2</sub> plants in group 8-15 were considered to belong to the dominant group. In the cross II-18-37×Black Mesdag no F<sub>1</sub> plants were in group 8-15. In the F<sub>1</sub> of II-18-4×Black Mesdag, 2 out of 6 plants were placed in group 8-15. The classification was corrected on the basis of the breeding behavior of F<sub>1</sub>, and in the first cross one-twelfth of the plants in hair group 8-15 were considered to be F<sub>1</sub> types and of low hair number, while one-third of the plants in group 8-15 of cross II-18-4×Black Mesdag were considered to be F<sub>1</sub> types. The classification after this correction is as follows:

Cross	Hair group		Deviation from 3:1	Probable error for 3:1	Deviation/P. E.
	Few	Many			
II-18-2×Black Mesdag F <sub>2</sub>	490	136	21	7.31	2.9
II-18-37×Black Mesdag F <sub>2</sub>	513	76	71	7.09	10.0
II-18-4×Black Mesdag F <sub>2</sub>	390	143	10	6.74	1.5

Two of the crosses could be satisfactorily explained on the basis of one main factor pair with a dominance of few hairs over many hairs, while in the cross of II-18-37×Black Mesdag the deviation from a 3:1 ratio is large.

To determine the genetic accuracy of the classification in  $F_2$  several lines were grown in  $F_3$  and studied for number of hairs on rachilla. The data for these  $F_3$  lines are given in Table 6.

TABLE 6.— $F_3$  breeding behavior for hairs on rachilla of certain  $F_2$  plants in cross of II-18-37  $\times$  Black Mesdag

Parent or $F_3$ cross	Average number of hairs on parent	Number of plants in groups, based on number of hairs on rachilla					
		0-7	8-15	16-23	24-31	32-39	Above
Black Mesdag		8	10	6	1		
II-18-37		25					
69-133 $F_3$	0.0	24	1				
-50 $F_3$	.0	25					
-113 $F_3$	.5	22	1	1			
-330 $F_3$	.7	17	6				
-47 $F_3$	1.0	20	4	1			
-1 $F_3$	2.8	23	2				
-5 $F_3$	4.2	23	2				
-31 $F_3$	6.3	10	6	6	1		
-46 $F_3$	8.5	19	2	5			
-7 $F_3$	11.3	11	5	6	3		
-250 $F_3$	12.8	3	4	4	8	4	2
-200 $F_3$	14.5	12	9	2	1		
-145 $F_3$	14.7	13	5	5	1	1	
-1550 $F_3$	14.3	7	11	4	2		1
-219 $F_3$	15.5	4	9	4	3	3	2
-1477 $F_3$	15.8	11	8	5	1		
-1536 $F_3$	16.2	5	7	3	5	4	1
-141 $F_3$	19.3	7	5	5	5	1	
-1470 $F_3$	21.3		2	8	6	5	4
-500 $F_3$	24.2		4	2	3	10	6
-12 $F_3$	26.1	3	2	2	2	10	6

The White Russian-Minota selection II-18-37 again bred true for few hairs and Black Mesdag proved more variable than in the preceding year, producing 8 out of 25 plants in the group 0-7. There was a general tendency for  $F_2$  plants with few hairs to produce progeny with a low hair number, and  $F_2$  plants with higher number of hairs on rachilla to produce progeny with a higher number. Some lines bred true for 0-7 hairs on the rachilla while some lines produced more hairs on the rachilla than the Black Mesdag parent.

#### AWN DEVELOPMENT

Inheritance of awn development was studied in a cross of Minota  $\times$  White Russian II-18-37 with Black Mesdag.

Black Mesdag has a rather strong awn on the lower grain in the spikelet, but lacks awns entirely on the second grain.

The awn, as usually found on the first grain, is rather thick and long, with a dark basal portion which is twisted to the left from the base. This dark portion is brownish black and mostly uniform in color but is sometimes striped by light-colored bands. The twisting and coloring extend usually about one-third of the whole length of the awns. The geniculation, or bending of the awns, seems to be caused directly by the twisting, which ceases suddenly at the bend. The degree of geniculation varies considerably from almost a right angle to a scarcely perceptible bend and seems to be proportionate to the degree of twisting and to the strength of the awn. There is much variation in the geniculation and strength of awns between different spikelets

and panicles on a single plant. The average percentage of awned spikelets in Black Mesdag was found to be 91.9, varying in 18 plants from 73.1 to 100 per cent.

Strain II-18-37 produced an average percentage of 2.1 awned spikelets, as determined by an examination of 30 plants. Only 7 of the 30 plants, however, produced any awns.

The groups for awn development were differentiated on the basis of percentage of awned spikelets. They were placed in five groups on this basis—Group 1, 81–100 per cent (the awning percentage similar to Black Mesdag); Group 2, 61–80 per cent; Group 3, 41–60 per cent; Group 4, 21 to 40 per cent; Group 5, 0–20 per cent (the awning percentage similar to White Russian×Minota II-18-37).

The  $F_1$  generation was intermediate both as to percentage and development of awns. Data on the parents and the  $F_1$  and  $F_2$  generations were compared. (See Table 7.)

TABLE 7.—*Inheritance of percentage of awned spikelets in a cross of Black Mesdag with a selection of White Russian×Minota II-18-37*

Parent or cross	Number of plants in groups as indicated *				
	Group 1	Group 2	Group 3	Group 4	Group 5
Black Mesdag.....	17	1	—	—	—
II-18-37.....	—	—	—	—	30
Black Mesdag×II-18-37 $F_1$ .....	2	6	3	1	3
Black Mesdag×II-18-37 $F_2$ .....	66	70	58	44	140

\* The groups were classified according to percentage of awned spikelets as follows: Group 1=81–100 per cent of spikelets awned; Group 2=61–80 per cent of spikelets awned; Group 3=41–60 per cent of spikelets awned; Group 4=21–40 per cent of spikelets awned; Group 5=0–20 per cent of spikelets awned.

The  $F_1$  was as variable as the  $F_2$  and gave all classes for percentage of awned spikelets. There was a large difference between the development of awns of the  $F_1$  and  $F_2$ , which can be demonstrated by a classification on the basis of geniculation and percentage of awned spikelets.

The classification of the parents and of the  $F_1$  and  $F_2$  generation is given in Table 8.

TABLE 8.—*Geniculation and percentage of awned spikelets in a cross of Black Mesdag with a selection of White Russian×Minota II-18-37*

Parent or cross	Number of plants in groups as indicated *						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Black Mesdag.....	17	1	—	—	—	—	—
II-18-37.....	—	—	—	—	—	23	7
Black Mesdag×II-18-37 $F_1$ .....	—	—	—	6	6	3	1
Black Mesdag×II-18-37 $F_2$ .....	66	21	12	57	58	105	59

\* The groups were classified as follows: Group 1=geniculate, 87–100 per cent of spikelets awned; Group 2=geniculate, 34–66 per cent of spikelets awned; Group 3=geniculate, 1–33 per cent of spikelets awned; Group 4=nongeniculate, 67–100 per cent of spikelets awned; Group 5=nongeniculate, 34–66 per cent of spikelets awned; Group 6=nongeniculate, 1–33 per cent of spikelets awned; Group 7=no spikelets awned.

Plants in Groups 1 to 3 contained geniculate awns, and the  $F_1$  showed a dominance of nongeniculate over geniculate, with some variability in the percentage of awned spikelets. There was a ratio in the  $F_2$  of 99 plants in Groups 1 to 3 to 279 with weak or no awns. Fifty-nine  $F_2$  plants produced no awns on the spikelet examined.

To determine the complexity of inheritance of the character, certain  $F_2$  plants were selected and their  $F_3$  progeny classified into seven groups for awn production, as in Table 9. It will be remembered that Groups 1 to 3 produce geniculate awns, Groups 4 to 6 nongeniculate awns, and that Group 7 produced no awns. The results of this study are presented in Table 9.

$F_2$  plants with 0 to few awns bred true or tended to breed true for this character in  $F_3$ . Eighteen of the twenty-nine nongeniculate  $F_2$  plants produced progeny with some geniculate plants in  $F_3$ , although nongeniculate habit was produced in much greater numbers than geniculate. Of the  $F_2$  geniculate plants which were tested by growing progeny in  $F_3$ , only 2 out of 11 tended to breed relatively true for geniculation. These two bred relatively true also for heavy percentage of awned spikelets.

TABLE 9.—Classification of  $F_2$  plants and  $F_3$  progeny on the basis of number and geniculation of awns

Classification of $F_2$ parent plant	Number of plants in $F_3$ progeny in groups as indicated *							Classification of $F_2$ parent plant	Number of plants in $F_3$ progeny in groups as indicated *						
	1	2	3	4	5	6	7		1	2	3	4	5	6	7
0 per cent.....						1	24	N, 55 per cent.....		1			8	11	5
Do.....						6	19	N, 58 per cent.....	1			1	2	19	7
Do.....					2	17	6	N, 60 per cent.....	2			2	6	15	
Do.....					6	11	8	N, 64 per cent.....	10	1		4	8	2	
Do.....					4	4	17	Do.....	2	2		5	8	7	1
N, 3 per cent <sup>b</sup> .....						3	22	Do.....	1				9	13	2
N, 5 per cent.....					1	8	16	N, 69 per cent.....	1			6	8	7	3
Do.....				1	4	6	14	N, 75 per cent.....	3			6	11	5	
N, 8 per cent.....		1			3	17	4	N, 79 per cent.....				1	6	18	
Do.....					1	14	10	N, 85 per cent.....				8	8	7	2
N, 11 per cent.....	3	3			3	13	3	N, 88 per cent.....	5			9	10	1	
N, 17 per cent.....	1	2		2	7	13	1	G, 20 per cent <sup>b</sup> .....	3	1		7	8	6	
N, 18 per cent.....				1	5	12	7	G, 31 per cent.....					9	12	4
N, 26 per cent.....		1			3	19	2	G, 29 per cent.....		4	1	1	4	13	2
N, 34 per cent.....			1	1	8	15		G, 48 per cent.....	1	3		1	8	11	1
N, 40 per cent.....					2	19	4	G, 52 per cent.....			1		12	12	
N, 41 per cent.....	2				2	19	2	G, 64 per cent.....	2	3		2	7	9	2
N, 44 per cent.....	2				8	14	1	G, 56 per cent.....	2	4			6	11	2
N, 46 per cent.....		3		2	8	10	2	G, 68 per cent.....		4	1	1	7	11	1
N, 48 per cent.....					2	21	2	G, 75 per cent.....				10	12	3	
N, 49 per cent.....					4	18	3	G, 98 per cent.....	21	1		3			
N, 52 per cent.....				2	19	4		G, 100 per cent.....	20	4			1		
N, 54 per cent.....	1			2	11	10	1								

\* The groups were classified as follows: Group 1=geniculate, 67-100 per cent of spikelets awned; Group 2=geniculate, 34-66 per cent of spikelets awned; Group 3=geniculate, 1-33 per cent of spikelets awned; Group 4=nongeniculate, 67-100 per cent of spikelets awned; Group 5=nongeniculate, 34-66 per cent of spikelets awned; Group 6=nongeniculate, 1-33 per cent of spikelets awned; Group 7, no spikelets awned.

<sup>b</sup> N=nongeniculate; G=geniculate.

The lack of agreement between  $F_2$  condition for awns and  $F_3$  breeding behavior indicates that environmental conditions probably exert a rather marked influence upon type. It would appear relatively easy, however, to obtain weak-awned types from such a cross, but the genetics of awn development appear to be a more difficult problem. Without doubt several genetic factors are involved.

## SMUT REACTION IN CROSS OF (WHITE RUSSIAN×MINOTA) II-18-37 WITH BLACK MESDAG

Random selections of  $F_2$  plants were made from the four groups—black, susceptible to stem rust; black, resistant to stem rust; white, susceptible; and white, resistant. Approximately 100  $F_3$  lines were grown from each group. The Minota-White Russian susceptible parent was grown about every 20 rows and the Black Mesdag only once.

The grain was smutted before being sown. There was no infection in Black Mesdag. The infection in the various lines, of which approximately 75 plants of each were grown, was determined by counting the number of infected plants and the number of panicles infected and noninfected in each. The percentage of infected plants was multiplied by the average percentage of infected panicles of the plants which gave some infection. The lines which were not infected in  $F_3$  in 1924 were harvested in bulk and tested in  $F_4$ . If they were again free from smut they were grown again in  $F_5$ . Lines with no infection were considered immune.

The lines of the smut-susceptible parent gave the following percentages of infection in 1924: 3.2; 4.6; 5.7; 6.4; 7.1; 7.9; 8.3; 8.4; 10.0; 10.8; 11.2; 12.4; 13.4; 15.0; 17.1. Immunity to smut in oats is dominant to susceptibility. If the same factors are involved, the susceptible  $F_3$  lines should be infected as severely as the Minota × White Russian parent. If only a single-factor pair is involved, the heterozygous lines should be expected to contain one-fourth as much infection on an average as the susceptible parent. One-fourth of 3.2 is 0.8, while one-fourth of 17.1 is 4.3, and one-fourth of 15.0 is 3.8. On this basis, on an average, 2 out of every 3  $F_3$  lines which are included in infection groups 3.2 to 4.3 should be heterozygous for smut and 1 should be homozygous for smut susceptibility. The separation in each group was made on this basis.

The detailed separation of the  $F_3$  hybrids with infection percentages between 3.2 and 4.3 is as follows: There were 11  $F_3$  lines in groups for smut infection 3.2–4.3 grown from  $F_2$  plants classified as black susceptible to rust. These were classed as susceptible and heterozygous for smut reaction as follows:

Four susceptible (*S*), with infection percentages 3.7; 4.0; 3.9; 4.3.

Seven heterozygous (*H1*), with infection percentages 3.4; 3.2; 3.2; 3.7; 3.3; 3.4; 3.7.

Eight  $F_3$  lines produced from  $F_2$  black-glumed plants resistant to rust were placed in susceptible and heterozygous smut classes on the same basis:

3 susceptible, with infection percentages 4.2; 4.1; 4.1.

5 heterozygous, with infection percentages 3.5; 4.0; 3.5; 3.4; 3.4.

Six  $F_3$  lines produced from  $F_2$  white-glumed plants susceptible to rust were classed as follows:

2 susceptible, with infection percentages 4.3; 4.0.

4 heterozygous, with infection percentages 3.8; 3.6; 3.4; 3.9.

Five  $F_3$  lines produced from  $F_2$  white-glumed plants, resistant to rust, were classified for smut as follows:

2 susceptible, 4.1; 4.1.

3 heterozygous, 3.4; 3.3; 4.0.



The classification of  $F_2$  plants on the basis of  $F_3$  to  $F_5$  breeding behavior (Table 10) was made as follows:

$S$ =as susceptible in 1924 as II-18-37 Minota  $\times$  White Russian, percentage of infection 4.4 or more.

$H1$ =heterozygous in 1924 with one-fourth as much smut, on an average, as the smut-susceptible parent. (The 30 lines with infection percentages 3.2 to 4.3 were distributed in these groups, 11 in  $S$  and the remainder in  $H1$ , as has been explained.)

$H2$ =0 infection in 1924, heterozygous with approximately one-fourth as much infection in 1925 as the susceptible parent.

$H3$ =heterozygous in 1924 with lower infection than 8 smutted panicles.

$H4$ =0 infection in 1924; about 10 panicles infected in 1925.

$R$ =0 in 1924, 1 to 4 panicles in 1925 or 1926.

$I$ =no infection in 1924 to 1926.

TABLE 10.—Classification of the  $F_3$  to  $F_5$  progeny of four groups of  $F_2$  plants on the basis of smut reaction

$F_2$ parent plant	Number of plants in classes for smut reaction						
	S	H1	H2	H3	H4	R	I
Black, resistant to rust.....	17	32	9	4	2	7	8
Black, susceptible to rust.....	21	36	8	7	3	5	6
White, resistant to rust.....	19	37	4	2	5	13	20
White, susceptible to rust.....	29	44	8	6	2	11	13
Total.....	86	149	29	19	12	36	47

The results of these studies prove that it is very easy to obtain immune lines from a cross of immune and susceptible. The differentiation between different classes of homozygous, susceptible, and heterozygous families is very difficult with a character like reaction to smut, which is not completely expressed in the progeny. The results indicate that there are separate factors which differentiate immunity and resistance, for numerous lines of Black Mesdag have been grown and no infection has been obtained. Lines which produce only a few infected panicles may be resistant. Susceptible and heterozygous lines unless extensively studied are difficult of separation.

Results similar to those obtained in these studies could be explained by two pairs of genetic factors,  $II$  and  $RR$  for immunity and resistance, respectively, located in Black Mesdag.  $I$  might be considered to be epistatic to  $R$ . It is impossible, however, to determine the exact genetic condition in these studies.

#### CORRELATION BETWEEN COLOR OF GLUMES AND OTHER CHARACTERS

##### COLOR OF GLUMES AND RUST REACTION

The parents of the crosses differed in several characters which furnished an opportunity to study linkage relations. A classification for the characters resistance versus susceptibility and glume color is given in Table II. Resistance versus susceptibility and black versus white glume color are each dependent on a single genetic factor pair, and these two pairs of factors appear to be independently inherited.

TABLE 11.—*Correlation between glume color classes and reaction to rust in the F<sub>2</sub> generation of three crosses between Black Mesdag and rust-resistant segregates from previous White Russian crosses*

Cross	Black resistant plants	Black susceptible plants	White resistant plants	White susceptible plants	Total number of individuals	X <sup>2</sup>	P
(White Russian×Victory) II-18-2×Black Mesdag, plant row No. 68:							
Obtained.....	477	158	148	51	834		
Calculated.....	469	156.5	156.5	52	834	0.632	Close to 1.
(Minota×White Russian) II-18-37×Black Mesdag, plant row No. 69:							
Obtained.....	2,182	725	780	268	3,935		
Calculated.....	2,213	738	738	246	3,935	3.286	0.3544
(Minota×White Russian) II-18-4×Black Mesdag, plant row No. 70:							
Obtained.....	781	288	268	84	1,421		
Calculated.....	799	266.5	266.5	89	1,421	2.430	.4947
All F <sub>2</sub> groups, plant row Nos. 68-70:							
Obtained.....	3,440	1,171	1,176	403	6,190		
Calculated.....	3,482	1,160.5	1,160.5	387	6,190	1.463	.6954

For plant row 68,  $X^2$  is only 0.632 and  $P$  consequently very close to 1. For plant row 69,  $X^2$  is somewhat higher, that is, 3.286, and  $P$  is 0.3544. For plant row 70,  $X^2=2.430$  and  $P=0.4947$ , and for the whole  $F_2$  population  $X^2=1.463$  and  $P=0.6954$ . Resistance versus susceptibility and black versus white glume color appear to be independently inherited.

#### COLOR OF GLUMES AND HAIRS ON RACHILLA

Two of the crosses, II-18-2 and II-18-4×Black Mesdag, could be satisfactorily explained on the basis of a single genetic factor pair for hairs on rachilla with nearly complete dominance of few over many hairs. It will be remembered that the Black Mesdag parent produces many hairs and that the white-glumed parents produce few hairs on the rachilla. The results given in Table 12 are on the basis of one-third as many white as black glumed segregates.

TABLE 12.—*Correlation between glume color and hairs on rachilla in F<sub>2</sub> crosses of a black-glumed many-haired variety with white-glumed few-haired strains*

Cross	Number of black-glumed plants having—		Number of white-glumed plants having—	
	Few hairs	Many hairs	Few hairs	Many hairs
II-18-2×Black Mesdag.....	329	101	117	26
II-18-4×Black Mesdag.....	206	85	55	14
Total.....	535	186	172	40

There is apparently a lower percentage of white-glumed plants with many hairs on rachilla than black glumed, which indicates a possible slight genetic linkage between the factors for glume color and hairs on the rachilla. Comparing the results obtained for the two crosses with a 9 : 3 : 3 : 1 ratio on the basis of  $X^2$  test of goodness

of fit, a value for  $P$  of 0.0858 was obtained. This means that on the basis of random sampling a deviation as great as the one obtained might be expected 858 times in 10,000 trials, or 1 in 11.7. This is not a wide deviation from expectation but here is a slight tendency for a linkage relation. Cross II-18-37  $\times$  Black Mesdag which deviated widely from a 3 : 1 ratio also produced more black-glumed many-haired segregates than white glumed. The percentage of many-haired segregates in the black and white glumed classes in this cross was 17.3 and 13.3 respectively. The results can be explained by the hypothesis of a loose linkage between the factors conditioning many versus few hairs and the factor pair for glume color.

#### COLOR OF GLUMES AND AWN DEVELOPMENT

The black and the white glumed  $F_2$  plants were classified for awn development into seven groups. These were:

- Group 1, geniculate awns, 67-100 per cent of spikelets awned.
- Group 2, geniculate awns, 34-66 per cent of spikelets awned.
- Group 3, geniculate awns, 1-33 per cent of spikelets awned.
- Group 4, nongeniculate awns, 67-100 per cent of spikelets awned.
- Group 5, nongeniculate awns, 34-66 per cent of spikelets awned.
- Group 6, nongeniculate awns, 1-33 per cent of spikelets awned.
- Group 7, no awns.

Black Mesdag was placed in Groups 1 and 2 and the few awned parents in Groups 6 and 7. The black and white glumed plants were placed in the seven awning classes as shown in Table 13:

TABLE 13.—Classification for awn development of black and white glumed plants

Color of glumes	Number of plants in groups for awn development							Total
	1	2	3	4	5	6	7	
Black.....	27	9	6	23	29	45	26	165
White.....	39	12	6	24	29	60	33	203

The double  $X^2$  test of goodness of fit was used to determine whether the two groups could be regarded as random samples drawn from the same population. A calculated  $X^2$  of 1.98 was obtained and a  $P$  of 0.9210, which indicates that the two samples could reasonably be considered random samples drawn from the same population. The results indicate no correlation or linkage between the factors for awn development and those for glume color.

#### COLOR OF GLUMES AND SMUT REACTION

The material was classified for smut reaction on the basis of  $F_3$  to  $F_5$  breeding behavior. The color of glumes of  $F_2$  plants was correlated with the breeding behavior for smut reaction. It will be remembered that the various lines were placed in several classes for smut reaction as follows:  $S$ , homozygous for smut susceptibility;  $H1$

\* The formula used was  $X^2 = S \left[ \frac{NN' \left( \frac{fp}{N} - \frac{fp'}{N'} \right)^2}{fp + fp'} \right]$ , where  $fp$  = frequency distribution of one sample,  $fp'$  = the frequency distribution of the other;  $S(fp) = N$  or total of one,  $S(fp') = N'$  or total of other.  $\frac{fp}{N}$ ,  $\frac{fp'}{N'} = fp$  and  $fp'$ , respectively, in percentage.

to *H*<sub>4</sub>, classes segregating for smut reaction and representing a progressive decrease in smut observed in classes *H*<sub>1</sub> to *H*<sub>4</sub>; *R*, resistant with only slight infection; *I*, immune from smut. The Black Mesdag parent was smut immune and the selections from the White Russian crosses used as the other parent were susceptible. The classification for color of glumes and smut reaction is given in Table 14.

TABLE 14.—*Classification of black and white glumed F<sub>2</sub> plants in relation to F<sub>2</sub> to F<sub>5</sub> breeding behavior of their progeny for smut reaction*

Color of glumes	Number of plants in groups for smut reaction						
	S	H1	H2	H3	H4	R	I
Black.....	38	68	17	11	5	12	14
White.....	48	81	12	8	7	24	33
Total.....	86	149	29	19	12	36	47

The double  $X^2$  test was used to determine whether similar smut reaction groups were obtained for the black and white segregates. A calculated  $X^2$  of 9.03 was obtained with a *P* of 0.1721. On this basis a worse agreement might be expected on the basis of random sampling 17 out of 100 trials. Probably therefore the segregation for smut reaction in the black and white glumed classes is of a similar nature.

## CORRELATION BETWEEN RUST REACTION AND OTHER CHARACTERS

### RUST REACTION AND HAIRS ON RACHILLA

Studies of correlation between reaction to stem rust and number of hairs on rachilla were made for the crosses of II-18-2 and II-18-4 with Black Mesdag which approached a monohybrid segregation for few versus many hairs. The results given in Table 15 are calculated on the basis of one-third as many susceptible as resistant plants, the classification for few versus many hairs being made as previously described.

It will be noted from the results that the agreement is good with a 9 : 3 : 3 : 1 ratio, which indicates that rust reaction and hairs on the rachilla of the second spikelet are independently inherited.

TABLE 15.—*Classification for reaction to rust and hairs on rachilla in the cross of Black Mesdag, a rust-susceptible, hairy-rachilla variety, with rust-resistant, few hairs on rachilla segregates of previous crosses of White Russian with Victory and Minota*

Cross	Number of resistant plants having—		Number of susceptible plants having—	
	Few hairs	Many hairs	Few hairs	Many hairs
II-18-2×Black Mesdag.....	162.7	43.0	53.0	17.2
II-18-4×Black Mesdag.....	141.6	61.0	48.9	18.6
Total.....	304.3	109.0	101.9	35.8

## RUST REACTION AND AWN DEVELOPMENT

F<sub>2</sub> plants resistant and susceptible to stem rust were selected at random from each of the four groups—black resistant, black susceptible, white resistant, white susceptible. As there was no apparent linkage between black versus white and groups for awn development, the resistant and susceptible plants can be compared directly without correction for the fact that selection of black and white glumed plants was made. The relation between rust reaction and awn development is given in Table 16.

TABLE 16.—*Correlation between rust reaction and awn development in the F<sub>2</sub> generation*

Rust reaction	Number of plants in groups for awn development						
	1	2	3	4	5	6	7
Resistant.....	26	12	4	24	31	51	31
Susceptible.....	40	9	8	33	27	54	28
Total.....	66	21	12	57	58	105	59

On the basis of the double  $X^2$  test the value of  $X^2$  was 5.4 and the value of  $P$  was 0.4956.

## RUST REACTION AND SMUT REACTION

F<sub>2</sub> plants were placed in two groups on the basis of reaction to rust—i. e., resistant and susceptible. F<sub>3</sub> to F<sub>5</sub> progeny selected at random from the four F<sub>2</sub> groups, black resistant, white resistant, black susceptible, white susceptible, were classified for smut reaction. As similar numbers of black resistant, and white resistant, black susceptible, and white susceptible plants were selected the smut reaction can be compared directly for the two groups for rust reaction. (See Table 17.)

TABLE 17.—*Correlated inheritance for rust and smut reaction*

F <sub>2</sub> plants for rust reaction	Number of plants in groups for F <sub>3</sub> to F <sub>5</sub> progeny for smut reaction				
	S	H1 and H2	H3 and H4	R	I
Resistant.....	38	82	13	20	28
Susceptible.....	50	96	18	16	19

The probability that these represent random samples from the same population was determined by the double  $X^2$  test.  $X^2=5.2$  and  $P=0.2697$ . No correlation between rust and smut reaction was obtained.

## CORRELATION BETWEEN HAIRS ON RACHILLA AND OTHER CHARACTERS

## HAIRS ON RACHILLA AND AWN DEVELOPMENT

Black Mesdag produced many hairs on the rachilla and the rust-resistant parents produced few. Similarly, Black Mesdag produced many awns and the rust-resistant parents few. In order to determine whether there was an association between the characters, development of awns and rachilla hairs, the  $F_2$  data from the cross of II-18-37×Black Mesdag were used and the correlation between the two groups of characters determined. The coefficient of contingency was used as a measure of correlation. (See Table 18.) The probable error of this coefficient was calculated from the formula  $P. E. \text{ of } C_1 \text{ or mean square contingency} = 2 \times 0.6745 \frac{1-C^2}{\sqrt{N}}$ . The calculated value of  $C_1 = 0.1867 \pm 0.0670$ . This indicates little or no correlation, and therefore segregation for awn development and hairs on rachilla appears to be independent.

TABLE 18.—Correlation between the development of awns and of hairs on the rachilla in the  $F_2$  generation of II-18-37×Black Mesdag

Classes for hairs on rachilla	Number of plants in groups for awn development							Total
	1	2	3	4	5	6	7	
0-7.....	57	17	9	43	44	88	53	311
8-15.....	7	2	3	12	10	12	4	50
16-23.....	2			2	4	5	1	14
24-31.....		2					1	3
Total.....	66	21	12	57	58	105	59	378

## HAIRS ON RACHILLA AND SMUT REACTION

The cross was between Black Mesdag, a smut-immune variety, which produces pubescence on the rachilla which supports the second kernel of the spikelet, and smut susceptible, weakly pubescent segregates of a previous White Russian cross. The  $F_2$  generation of the cross of II-18-37×Black Mesdag was used to determine the correlation, if any, between smut reaction and production of hairs on the rachilla. (See Table 19.)

The calculated coefficient of contingency for these results was  $0.0435 \pm 0.0692$ , which indicates that smut reaction and hairs on rachilla are independent in inheritance.

TABLE 19.—Correlation between number of hairs on the rachilla which supports the second kernel of the spikelet of  $F_2$  generation plants and smut reaction, as determined from their  $F_3$  to  $F_5$  generation progeny

Classes for hairs on rachilla	Number of plants in smut-reaction classes					Total
	I	R	H3 and H4	H1 and H2	S	
0-7.....	40	30	28	150	63	311
8-15.....	6	3	2	20	19	50
16-23.....	1	2	1	6	4	14
24-31.....		1				3
Total.....	47	36	31	178	86	378

## CORRELATION BETWEEN AWN DEVELOPMENT AND SMUT REACTION

The Black Mesdag or immune parent produces many strongly developed awns while the smut-susceptible Minota  $\times$  White Russian parent, II-18-37, produces a few weakly developed awns.  $F_2$ -generation plants were classified into seven groups for development and geniculation of awns as has been described.

A correlation table for awn development in  $F_2$  and smut reaction as determined from  $F_3$  to  $F_5$  data is presented in Table 20.

TABLE 20.—Correlation between awn development of  $F_2$  plants and smut reaction of their  $F_3$  to  $F_5$  progeny

Smut reaction	Number of plants in $F_2$ groups for awn development							
	1	2	3	4	5	6	7	Total
I.....	9	3	2	5	6	18	4	47
B.....	9	2	1	5	3	7	9	36
H3 and H4.....	7	3	1	5	6	8	1	31
H1 and H2.....	31	12	7	21	27	51	20	178
S.....	10	1	1	21	16	21	16	86
Total.....	66	21	12	57	58	105	59	378

The coefficient of contingency for awn development and smut reaction was  $C_1 = 0.1121 \pm 0.0685$ . No close association or linkage is indicated.

## SUMMARY

A correlated study of the mode of inheritance of reaction to black stem rust, *Puccinia graminis avenae*, and loose and covered smuts, *Ustilago avenae* and *U. levis*, in relation to other differential characters was made.

The rust-resistant parents were segregates from previous crosses of White Russian with Victory or Minota. Three selections were used, "Anthony," which was selected from a cross of White Russian  $\times$  Victory and which bore the Nursery Series Number II-18-2, and two unnamed selections from crosses of White Russian with Minota which bore the Nursery Series No. II-18-4 and II-18-37. Nursery Series No. II-18-37 was used most extensively in the studies. These three selections produced white glumes, weak awns, few to no hairs on the second kernel of the spikelet and were susceptible to smut. The smut-immune parent, Black Mesdag, produced black glumes, strongly developed, geniculate awns, many hairs on the rachilla of the second spikelet, and was rust susceptible.

Black versus white glumes and rust resistance versus susceptibility gave close approximations to 3 : 1 segregations with black dominant over white and rust resistance dominant over susceptibility.

Segregation for number of hairs on the rachilla of the second spikelet agreed well with a 3 : 1 ratio in  $F_2$  of few to many hairs on the spikelet for the crosses of II-18-2 and II-18-4 with Black Mesdag. The cross of II-18-37 and Black Mesdag produced a greater proportion of few-haired segregates than would be expected on the basis of a 3 : 1 ratio.

Segregation for number and strength of awns occurred, and the classification of  $F_2$  plants was compared with  $F_3$  breeding behavior. The results could not be explained on any simple genetic basis. There were 66  $F_2$  plants out of 378 in the cross of II-18-37  $\times$  Black Mesdag which were classified in the awnless group. Five of these were selected and their progeny grown in  $F_3$ . These bred true for none to few weak awns. The geniculate, strongly awned  $F_2$  types, however, segregated in  $F_3$  for the most part.

Approximately the same number of  $F_2$  plants were selected at random from each of the four groups—black, resistant to rust; black, susceptible to rust; white, resistant; and white, susceptible, and their progeny tested in  $F_3$  to  $F_5$  for smut reaction. Of a total of 378 lines, 86 were classed as susceptible on the basis of  $F_3$  data. These were as susceptible as the II-18-37 parent. Lines which produced no smut in  $F_3$  to  $F_5$  were classed as immune. There were 47 of these lines. Some lines appeared highly resistant and only produced a small percentage of infection. Thirty-six lines were placed in this class. The remainder of the lines, of which there were 209, produced some smut infection although less than the susceptible parent and more than the lines classed as resistant. The susceptible parent produced a small percentage of infection, although each row tested showed some smut each year. The genetic factors for smut reaction can not be determined with accuracy in these studies. Results of the same general nature received here could be obtained by two factors  $I$  and  $R$  for immunity and resistance, respectively, both carried in the Black Mesdag parent, each allelomorphic to factors for susceptibility with the further hypothesis of  $I$  epistatic to  $R$ . This however, is suggested only as a possible explanation.

Black versus white glumes appeared to be independently inherited from resistance versus susceptibility to rust.

The two classes for color of glumes in  $F_2$  black and white were compared for segregation of awns and for smut reaction by the double  $X^2$  test of goodness of fit.  $P$  values of 0.9113 and 0.2226 were obtained, which indicates no linkage between factors for color of glumes and for awn development and smut reaction.

There was a slight tendency in all three crosses for a loose linkage between genetic factors for hairs on rachilla and for glume color, although the linkage, if any, was very loose.

No correlation between rust reaction and awn development or rust reaction and smut reaction could be demonstrated by the  $X^2$  test.  $P$  values of 0.2718 and 0.2697 were obtained. A worse fit could be expected on the basis of random sampling 27 times out of 100 or 1 out of 4.

The coefficient of contingency was used to determine any possible linkage between hairs on rachilla and awn development, hairs on rachilla and smut reaction, and smut reaction and awn development. All three calculated coefficients were low and hardly significant in the light of their probable errors.

From these studies several homozygous lines have been obtained which are resistant to black stem rust, immune from smut, and which have weak awns and white glumes. These are being compared with Victory and Minota for yielding ability.



## LITERATURE CITED

- (1) BARNEY, A. F.  
1924. THE INHERITANCE OF SMUT RESISTANCE IN CROSSES OF CERTAIN VARIETIES OF OATS. *Jour. Amer. Soc. Agron.* 16: 283-291, illus.
- (2) COFFMAN, F. A., PARKER, J. H., and QUISENBERRY, K. S.  
1925. A STUDY OF VARIABILITY IN THE BURR OAT. *Jour. Agr. Research* 30: [1]-64, illus.
- (3) FRASER, A. C.  
1919. THE INHERITANCE OF THE WEAK AWN IN CERTAIN AVENA CROSSES AND ITS RELATION TO OTHER CHARACTERS OF THE OAT GRAIN. *N. Y. Cornell Agr. Expt. Sta. Mem.* 23, p. 635-676, illus.
- (4) GAINES, E. F.  
1925. THE INHERITANCE OF DISEASE RESISTANCE IN WHEAT AND OATS. *Phytopathology* 15: [341]-349.
- (5) GARBER, R. J.  
1922. INHERITANCE AND YIELD WITH PARTICULAR REFERENCE TO RUST RESISTANCE AND PANICLE TYPE IN OATS. *Minn. Agr. Expt. Sta. Tech. Bul.* 7, 62 p., illus.
- (6) GRIFFEE, F.  
1922. BREEDING OATS RESISTANT TO STEM RUST. *Jour. Heredity* 13: 187-190, illus.
- (7) ——— and HAYES, H. K.  
1925. NATURAL CROSSING IN OATS. *Jour. Amer. Soc. Agron.* 17: 545-549.
- (8) HAYES, H. K., and GARBER, R. J.  
1927. BREEDING CROP PLANTS. Ed. 2, 438 p., illus. New York and London, McGraw-Hill Book Co., Inc.
- (9) LOVE, H. H., and CRAIG, W. T.  
1918. THE RELATION BETWEEN COLOR AND OTHER CHARACTERS IN CERTAIN AVENA CROSSES. *Amer. Nat.* 52: 369-383.
- (10) NILSSON-EHLE, H.  
1911. KREUZUNGSUNTERSUCHUNGEN AN HAFER UND WEIZEN. *Lunds Univ. Årsskr. (N. F.) Afd. 2, Bd. 7, No. 6*, 82 p.
- (11) REED, G. M.  
1920. VARIETAL RESISTANCE AND SUSCEPTIBILITY OF OATS TO POWDERY MILDEW, CROWN RUST, AND SMUTS. *Missouri Agr. Expt. Sta. Research Bul.* 37, 41 p., illus.
- (12) ———  
1924. PHYSIOLOGIC RACES OF OAT SMUTS. *Amer. Jour. Bot.* 11: 483-492, illus.
- (13) ——— and STANTON, T. R.  
1925. RELATIVE SUSCEPTIBILITY OF SELECTIONS FROM A FULGHUM-SWEDISH SELECT CROSS TO THE SMUTS OF OATS. *Jour. Agr. Research* 30: 375-391, illus.
- (14) STAKMAN, E. C., LEVINE, M. N., and BAILEY, D. L.  
1923. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP. *Jour. Agr. Research* 24: 1013-1018, illus.
- (15) WAKABAYASHI, S.  
1921. A STUDY OF HYBRID OATS, AVENA STERILIS × AVENA ORIENTALIS. *Jour. Amer. Soc. Agron.* 13: 259-266.



# VARIATIONS OF THE COLLOIDAL MATERIAL IN TYPICAL AREAS OF THE LEONARDTOWN SILT LOAM SOIL<sup>1</sup>

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## INTRODUCTION

Previous studies in this bureau have shown that the behavior of soil in the laboratory is governed very largely by the kind and quantity of colloidal material present (4, 15)<sup>2</sup>. It has also been pointed out that the character of the colloidal material should be considered in soil classification (9), since the colloid is distinctively the weathered product of the soil. Information as to the kinds of colloid present in the various cultivated areas is therefore important; and as a preliminary step toward obtaining such data, a study has been made of the colloidal materials present in various areas that have been mapped as a single type of soil.

Data have already been obtained regarding the composition and properties of the colloids present in samples collected from many types of soil (18, 10). These data, collected to show variations of the colloidal soil material in soils in general, can not safely be taken as showing the kinds of colloidal material that are characteristic of the particular types of soil sampled, since none of the types was represented by more than two samples, and the variability of a soil type with respect to colloidal material is unknown. Obviously, unless the colloidal material is fairly uniform in different samples of a type of soil, the nature of the colloid can not be regarded as one of the characteristics of soils as now classified. A priori there is no reason why the colloidal material should be especially uniform throughout an area occupied by a particular type of soil, since the character of the colloidal material is not one of the criteria used in classifying soils. On the other hand, the nature of the colloidal material plays such an important part in determining nearly all properties of a soil that it might be expected to vary little in areas of soil whose characteristics are so nearly uniform as different areas of a single soil type.

The results reported in this paper indicate the variation that may be expected in colloidal materials isolated from different samples of a fairly well-defined type of soil. The data given concern the chemical composition and properties of the colloidal material present in samples of Leonardtown silt loam collected in scattered areas where this type of soil appears well developed.

## REVIEW OF LITERATURE

Apparently no studies have been made dealing specifically with variation of the colloidal soil material within a soil type. However, analyses made by several investigators of the clay or colloidal mate-

<sup>1</sup> Received for publication Nov. 22, 1927; issued May, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 470.

rial isolated from different soils give some indication of the variation that may be expected.

It has previously been pointed out that, although colloids from different soils may differ widely in composition, samples of different soils within a restricted climatic region often contain very similar colloids (18). Analyses reported by Hall and Russell (12) indicate that four fertile soil types in southeastern England contain clays of approximately the same composition, and three less fertile types from the same regions contain clays which differ very little. Clay fractions of 10 North Wales soil samples reported by G. W. Robinson (16) are less uniform in composition. The difference between the highest and lowest silica content of the 10 clays is about 15 per cent of the average content, and the extreme variations of aluminum and iron oxides are, respectively, 34 and 38 per cent of the averages of these constituents. These 10 samples represent three soil types as defined by Robinson. Different samples of a single type show about half these variations. Bradfield (6) found the colloidal materials present in subsoils of 11 different soils in Missouri to be fairly uniform in composition. Differences between the highest and lowest silica and alumina contents were, respectively, 8 and 23 per cent of the average quantities present in the 11 samples. Analyses reported by Robinson and Holmes (18) of colloidal materials isolated from 45 soil and subsoil samples taken in different parts of the United States include 10 colloids that are fairly uniform in composition. Differences between the highest and lowest silica or alumina contents are only about 8 per cent of the average for these samples, whereas differences between the extremes of  $\text{Fe}_2\text{O}_3$  or between extremes of combined water are about 20 per cent of the average. It was pointed out as significant that most of these samples are from Maryland—a comparatively restricted area.

The preceding data indicate that in certain regions different types of soil contain colloids which do not differ widely in their major constituents. One would therefore expect a single type of soil to be quite uniform with respect to the kind of colloidal material present.

#### DESCRIPTION OF SAMPLES

The type of soil examined for variability of the colloidal material was the Leonardtown silt loam. This is a soil of very pronounced characteristics and is therefore fully as well defined as the average soil. Its characteristics, as described by C. F. Marbut in a memorandum to the author, are as follows:

In the uncultivated Leonardtown silt loam, the features characteristic of the timbered soils of the eastern United States are well developed. The A horizon is usually about 10 inches thick. The first half inch is colored dark by decomposing forest debris and the remainder of A is pale yellow to almost gray. The structure is single-grained with lamination well developed. The upper part of the B horizon, usually 10 to 15 inches thick, is yellowish with a faint reddish brown shade. The nut structure of the particles is so highly developed that the material falls into pieces when at the optimum water content. The outside of the structure particles is lighter in color than the inside, but there is no gray coating. Although the texture is heavier than that of the A horizon, there is no induration. The lower part of the B horizon, beginning usually at the eighteenth to the twenty-fourth inch, is entirely different from upper B. It is so highly indurated that penetration with a soil auger is difficult, and on drying it becomes as hard as sandstone. It is predominantly brownish gray in color, but the colors are unevenly distributed, some spots being gray. It contains many irregularly shaped pores, the wall of which are lined with a brownish coating. The C horizon

beneath the indurated B<sub>2</sub> layer is somewhat variable in character, ranging from a reddish clay to a gravelly clay. In no case is it indurated or cemented.

This type of soil occupies level or nearly level areas in the coastal plain of eastern Maryland and Virginia.

The purpose of this work was not to determine the average composition of the colloidal material in the whole area of Leonardtown silt loam that has been mapped. This study was restricted to determining variability in the colloidal material of soil samples taken in separated areas of Leonardtown silt loam in which the profile is typically developed. The results thus show the variability found in typical Leonardtown silt loam spots and not in all the areas that have been mapped as Leonardtown silt loam.

Three areas near one another and five widely separated were chosen for sampling; otherwise the samples were taken virtually at random. No attempt was made to obtain samples of exceptional uniformity by preliminary examination and rejection of samples. The only precaution observed in selecting a spot for sampling was to see that the profile had well-developed Leonardtown characteristics. The soil samples were not composites but were taken from single holes. The vertical section of soil included in the actual sample thus had a surface area of only a few square inches.

Data regarding the soil samples from which colloidal material was isolated are given in Table 1.

TABLE 1.—*Situation, topography, and vegetation of localities in which samples were collected; horizon and depth from which samples were taken; and colloidal material in samples, estimated by water adsorption and actually isolated from samples*

Locality No.	Locality in which samples were collected	Topography	Vegetation	Horizon	Depth of sample	Colloidal material in sample estimated by water adsorption	Colloidal material actually isolated from sample
1	2 miles northwest of Forestville, Prince Georges County, Md.	High, flat.....	Old grass field cover.	A	Inches 0-7	Per cent 10.5	Per cent 9.1
2	Do..... 15 feet south of locality No. 1.	High, flat.....	Old grass field cover.	B <sub>1</sub> B <sub>1</sub>	7-17 7-17	25.0 26.6	21.0 21.3
3	200 yards south of locality No. 1.	.....do.....	.....do.....	A	0-7	10.9	9.4
4	1½ mile north of Meadows, Prince Georges County, Md.; 2 miles south of locality No. 1.	Low, flat.....	Forest.....	A	0-12	10.7	7.3
	Do.....	.....	.....	B <sub>1</sub>	12-18	26.1	22.5
	Do.....	.....	.....	B <sub>2</sub>	18-28	11.5	10.3
5	½ mile southwest of Mattawoman, Charles County, Md.; 14 miles south of locality No. 1.	Low, flat.....	Forest.....	A	0-7	11.9	8.8
6	Do..... ½ mile west of Middleton, Charles County, Md.; 18 miles south of locality No. 1.	Low, flat.....	Forest.....	A	0-7	25.4 8.9	18.0 7.5
7	Do..... 1¼ miles west of Laurel, Prince Georges County, Md.; 19 miles north of locality No. 1.	High, flat.....	Old pasture-land cover.	B <sub>1</sub> A	7-14 1-8	18.0 12.0	14.8 9.7
	Do.....	.....	.....	B <sub>1</sub>	8-18	26.1	23.9
	Do.....	.....	.....	B <sub>2</sub>	19-30	15.0	13.6
8	1¼ miles south of Laurel, Prince Georges County, Md.; 17 miles north of locality No. 1.	High, slightly rolling.	Old grass-field cover.	A	1-8		
	Do.....	.....	.....	B <sub>1</sub>	8-18	25.9	23.1

It will be seen that in some cases colloidal material was isolated from three horizons in the profile, but in most cases the examination was limited to the A and upper B horizons.

### METHODS

The colloidal material was isolated from the soil very much as described in a previous publication (10), except that the distilled water used in the first dispersion of the soil was recovered and used for subsequent treatments of the sample. The water was maintained at a  $P_H$  of about 8 by the addition of a few drops of ammonia when necessary. The colloidal material isolated was obviously a fair sample of that in the soil, since the greater part was isolated in each case. The quantities of colloid isolated and the quantities present in the soil as shown by the water vapor absorbed by the soil over 3.3 per cent sulphuric acid are shown in columns 8 and 7 of Table 1. It has been shown that this estimation of the total quantity of colloid is fairly accurate in the case of most soils (10).

Analysis of the colloidal material was made by the fusion method. The procedure in most respects was similar to that employed by the Association of Official Agricultural Chemists (5), but determinations of  $TiO_2$ ,  $MnO$ , and  $P_2O_5$  were made in accord with Hillebrand's methods for the analysis of silicate rocks (13). Organic matter was determined by combustion in an electric furnace, the  $CO_2$  evolved being calculated to organic matter by the factor 0.471. The water of combination was taken to be the difference between the ignition loss and organic matter.

Hydrogen-ion determinations were made electrometrically with a hydrogen electrode essentially as described by Gillespie (11). The hydrogen-ion values of the isolated colloids were not determined, since they were altered by the ammonia used in the process of isolation. Determinations, however, were made of the untreated soil. These may be taken as representing approximately the hydrogen-ion concentrations of the unaltered colloid materials, since the  $P_H$  of a soil is primarily that of the colloidal material.

The quantity of water vapor adsorbed by the whole soil over 3.3 per cent (by weight) sulphuric acid was determined for the purpose of estimating the quantity of colloid in the soil. The method used has been described in a previous publication (10). Determinations were also made of the water vapor adsorbed by the isolated colloids over 30 per cent acid for the purpose of showing differences in the materials. Previous studies showed that different soil colloids adsorb similar quantities of water vapor over 3.3 per cent acid (17), but widely different quantities from the drier atmosphere afforded by 30 per cent acid (3).

The quantity of ammonia gas adsorbed by the colloidal material was determined by the method described in a previous publication of this bureau (10).

The quantity of barium adsorbed from a normal  $BaCl_2$  solution was taken as a measure of total base-exchange capacity of the colloidal material. The determination was conducted as follows: 4 gm. of colloidal material were thoroughly agitated with 100 c. c. of a normal solution of  $BaCl_2$  which had been adjusted to  $P_H$  7. After standing overnight the mixture was filtered, and the colloidal material was washed on the filter with successive portions of the  $BaCl_2$  solution until the total filtrate amounted to 500 c. c. In order to remove the

excess  $\text{BaCl}_2$ , the colloidal material was then washed with distilled water until the filtrate gave no test for chlorides. The adsorbed barium was extracted from the colloid by washing with approximately 700 c. c. of  $n/20$   $\text{HCl}$ . Complete extraction of the barium was determined by testing the final washings for barium. The barium in the filtrate, representing the quantity originally adsorbed, was determined as sulphate.

#### COMPOSITION AND PROPERTIES OF COLLOIDAL MATERIALS PRESENT IN THE DIFFERENT SOIL SAMPLES

The composition of the colloids isolated from the different samples of soil is given in Table 2. In this table the percentages of most constituents are calculated on the weight of total inorganic material (i. e., weight of oven-dried samples minus organic matter) rather than on the weight of the whole sample. Organic matter varies considerably in different samples, and when this is eliminated the percentages of the inorganic constituents become more constant.

In order that variations in the different constituents may be readily compared, the standard deviation and coefficient of variability are given for each constituent. These two constants are calculated by the usual formulas—standard deviation,  $\sigma = \sqrt{\frac{\sum d^2}{N}}$ , and coefficient

of variability,  $C = \frac{\sigma}{M} \times 100$ . In these formulas,  $d$  represents the deviation of each determination from the mean,  $M$ , of the whole number of determinations,  $N$  (7).

Apparently, variation in composition of the colloidal material in different localities is shown by samples from either the A or  $B_1$  horizon, since the colloids in these two horizons have very nearly the same chemical composition when the results are calculated on an inorganic basis. Only organic matter and constituents associated with it, such as ignition loss and possibly  $\text{SO}_3$  and  $\text{P}_2\text{O}_5$ , differ appreciably in the colloids from these two horizons. The colloidal material in the  $B_2$ , or hardpan horizon, also seems similar to that of the A and  $B_1$  horizons. This is certainly true of the profile taken in locality No. 7. In view of the similarity of the colloidal materials in the A and B horizons, any variation found in the colloids of different localities is evidently not due to failure to distinguish sharply between the A and B horizons in sampling the various soil profiles.

Colloidal materials of Leonardtown silt loam from the different localities show considerable variation in organic matter and in those constituents which are present in small quantity—less than approximately one-half of 1 per cent.<sup>3</sup> In organic matter,  $\text{SO}_3$  and  $\text{P}_2\text{O}_5$ , the colloids show almost as wide variation as the colloids which were isolated from many different soils in a previous investigation (18); hence these constituents do little toward distinguishing the colloids of Table 2 as coming from a particular kind of soil or group of soils. Manganese and lime are also quite variable, but the percentages of these constituents should not be regarded as wholly uncharacteristic

<sup>3</sup> The exact degree of variation in  $\text{CaO}$ ,  $\text{Na}_2\text{O}$ ,  $\text{P}_2\text{O}_5$ , and in  $\text{SO}_3$  is somewhat uncertain, since in many cases the analytical error is appreciable as compared with the quantities present. But even after a liberal allowance is made for analytical error, it is evident that the samples as a whole are quite variable in those constituents which are minor in quantity. Practically all the differences shown in  $\text{MnO}$  are real, since this determination is accurate within about two-thousandths of 1 per cent.

TABLE 2.—Analyses of colloidal materials isolated from samples of Leonardtown silt loam

Sample from—	Locality No.	Horizon	Constituents expressed as percentage of the inorganic material (oven-dried colloid minus organic matter)											Constituents expressed as percentage of whole oven-dried colloid including organic matter		Molecular ratio $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	
			SiO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	Combined H <sub>2</sub> O	Ignition loss		Organic matter
		A	42.56	1.04	12.69	27.46	0.07	0.57	1.06	1.46	0.40	0.46	0.80	11.95	23.54	13.16	2.03
		A	43.68	.86	10.80	28.50	.03	.22	.76	1.39	.29	.10	.90	12.82	20.27	8.44	2.08
		A	40.44	.73	10.92	31.01	.03	.23	.88	1.50	.43	.21	.40	12.68	20.26	8.55	1.80
		A	43.20	.87	12.22	27.70	.08	.47	.89	1.34	.43	.26	.46	11.98	16.30	4.92	2.05
		A	43.22	.86	12.55	28.51	.06	.46	.78	1.35	.44	.22	.46	11.70	17.10	6.09	2.03
		A	43.26	.82	11.06	28.18	.08	.33	1.42	1.42	.12	.44	.44	11.20	16.81	6.23	2.15
		A	43.80	.94	12.60	28.54	.08	.43	.97	1.51	.24	.51	.44	10.83	16.22	6.24	2.02
		Mean	43.15	.90	11.82	28.56	.06	.39	.87	1.42	.35	.32	.50	11.91	18.65	7.66	2.02
		Standard deviation	1.35	.09	6.79	1.07	.02	.12	.11	.06	.13	.15	.24	.68	2.57	2.49	5.0
		Coefficient of variability	3.1	10.0	6.7	3.7	33.3	30.8	12.6	4.2	37.1	46.9	48.0	5.7	13.8	32.5	
		B <sub>1</sub>	42.84	.84	13.50	27.83	.04	.40	1.06	1.31	.36	.19	.50	11.49	15.74	4.80	1.99
		B <sub>1</sub>	42.84	.95	13.05	27.53	.06	.37	.88	1.30	.30	.20	.33	11.36	14.74	3.82	1.98
		B <sub>1</sub>	43.67	.81	12.36	27.73	.03	.21	.88	1.32	.24	.09	.30	11.58	13.67	2.37	2.07
		B <sub>1</sub>	43.25	.86	13.25	27.30	.02	.36	.68	1.05	.37	.15	.18	12.60	14.01	1.70	2.05
		B <sub>1</sub>	42.65	.79	13.69	27.85	.05	.24	.76	1.04	.52	.24	.14	12.98	15.58	2.98	1.98
		B <sub>1</sub>	43.75	.79	14.35	27.00	.02	.14	.92	1.42	.26	.18	.19	10.86	12.68	1.91	2.05
		B <sub>1</sub>	43.00	.86	14.15	28.15	.07	.40	.84	1.43	.23	.24	.18	10.83	12.89	2.31	1.97
		Mean	43.14	.84	13.60	27.63	.04	.30	.86	1.27	.33	.18	.26	11.66	14.19	2.84	2.01
		Standard deviation	.40	.05	.48	.36	.02	.10	.11	.15	.09	.05	.12	.75	1.3	1.03	.04
		Coefficient of variability	.9	6.0	3.5	1.3	40.0	33.3	12.8	11.8	27.3	27.8	46.2	6.4	9.2	36.3	2.0
		B <sub>2</sub>	46.68	.95	7.75	32.34	.01	.26	.50	.68	.20	.17	-----	11.71	12.88	1.33	2.13
		B <sub>2</sub>	44.15	.92	12.97	29.09	.01	.51	1.06	1.30	.20	.12	-----	10.65	12.27	1.82	2.07



of the Leonardtown soil, since the Leonardtown colloids are uniformly lower in these constituents than many other soil colloids that have been analyzed.

In  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ ,  $\text{K}_2\text{O}$ ,  $\text{MgO}$ , and combined water, the colloids from the different localities vary little. The coefficients of variability range from 0.9 for  $\text{SiO}_2$  to 12.8 for  $\text{MgO}$ . The most constant constituents are  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ , and combined water, the standard deviations being approximately 5 per cent of the means, as shown by the coefficients of variability. These four constituents make up about 95 per cent of the inorganic material and the  $\text{TiO}_2$ ,  $\text{K}_2\text{O}$ , and  $\text{MgO}$  account for an additional 3 per cent. Evidently, then, so far as the bulk of the inorganic material is concerned, the different samples of colloidal material are fairly uniform in composition.

In order to characterize further the different samples of colloidal material, some of the more important properties were determined. These included the water vapor adsorbed over 30 per cent sulphuric acid, the quantity of ammonia gas adsorbed at a pressure of 1 atmosphere, the base-exchange capacity as measured by the barium adsorbed from a normal  $\text{BaCl}_2$  solution, and the hydrogen-ion concentration. Previous studies show that different colloidal soil materials may vary widely in these properties (10, 3). The procedure followed in these determinations is described on page 462.

It should be pointed out that determinations of these properties are probably less reliable measures of variability in the colloid samples than the chemical composition, since it is quite possible that some of the properties of the colloids were altered by the procedure of isolation. Previous studies show that in the isolation of certain colloids the heat of wetting and the adsorptive capacity are altered appreciably (1, 10). Doubtless, in the case of these samples there was some alteration in the capacities for adsorbing water, ammonia, and barium; but since the colloidal materials were all very much alike and were isolated by the same procedure, it is to be presumed that they were all altered to approximately the same extent. Also, too much importance should not be attached to the  $P_H$  determination as an indication of variability, since this determination was made on the whole soil, and it may have been affected somewhat by the noncolloidal constituents of the soil. The results of this group of determinations are given in Table 3.

It is apparent from Table 3 that the colloids from the different localities are fairly constant in four important properties. In hydrogen-ion concentration and in adsorptive capacity for water vapor and ammonia, the variability is about the same as that shown by the four major chemical constituents ( $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ , and combined water). The variability in base-exchange capacity (Ba adsorbed) is somewhat greater; it is about the same as the variability in  $\text{K}_2\text{O}$ ,  $\text{MgO}$ , and  $\text{TiO}_2$ .

Thus far, variability of the colloid samples has been judged from the variation in separate constituents and single properties. Obviously, this variability of single constituents does not show how constant the samples are in their whole composition, since one sample may be decidedly different from the other samples in one or two constituents but quite similar in all others. A better idea of how constant the samples are, taking everything into consideration, may

be gained from Table 4. In this table the quantities of the more constant chemical constituents and the values for four properties are expressed relative to the means of the A and B<sub>1</sub> colloids.

TABLE 3.—*Properties of the different samples of colloidal material*

Samples from—		Quantity of water vapor adsorbed per 100 gm. of colloid	Quantity of NH <sub>3</sub> gas adsorbed per 100 gm. of colloid	Quantity of Ba adsorbed per 100 gm. of colloid	Hydrogen-ion concentration of untreated soil
Locality No.	Horizon				
		Gm.	Gm.	Milli-equivalents	P <sub>H</sub>
4.....	A	5.69	2.69	2.30	4.6
5.....	A	5.50	2.45	2.10	4.6
6.....	A	5.11	2.52	1.85	4.6
7.....	A	5.37	2.56	1.51	4.5
8.....	A	5.73	2.85	2.11	4.5
Mean.....		5.48	2.61	1.98	4.6
Standard deviation.....		.21	.14	.27	.02
Coefficient of variability.....		3.8	5.4	13.6	.4
1.....	B <sub>1</sub>	7.06	2.65	2.60	4.9
2.....	B <sub>1</sub>	7.41	2.86	2.55	4.6
4.....	B <sub>1</sub>	7.43	2.97	2.33	4.5
5.....	B <sub>1</sub>	6.68	2.56	2.15	4.9
6.....	B <sub>1</sub>	6.33	2.67	2.15	4.7
7.....	B <sub>1</sub>	6.61	2.76	2.38	5.0
8.....	B <sub>1</sub>	7.24	2.91	2.33	4.9
Mean.....		6.97	2.77	2.36	4.8
Standard deviation.....		.40	.14	.16	.17
Coefficient of variability.....		5.7	5.1	6.8	3.5
4.....	B <sub>2</sub>	5.54	2.64	2.55	5.1
7.....	B <sub>2</sub>	9.29	2.85	3.41	4.7

TABLE 4.—*Composition and adsorptive capacity of the various samples, expressed relative to the means as 100*

Sample from—		The more constant chemical constituents of the colloid								Adsorption capacity of the colloid for—			P <sub>H</sub>
Locality number	Horizon	SiO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	MgO	K <sub>2</sub> O	Combined H <sub>2</sub> O	Mols. $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3+\text{Fe}_2\text{O}_3}$	Water vapor	Ammonia gas	Ba from BaCl <sub>2</sub> solution	
1.....	A	99	116	107	96	122	103	100	100	-----	-----	-----	-----
3.....	A	101	96	91	100	87	98	103	103	-----	-----	-----	-----
4.....	A	94	81	92	109	101	106	108	89	104	103	112	100
5.....	A	100	97	103	97	102	94	101	101	100	94	107	100
6.....	A	100	106	106	100	90	95	98	100	105	109	107	98
7.....	A	105	102	97	99	87	100	95	106	98	98	94	100
8.....	A	101	104	107	100	111	107	91	100	105	109	107	98
1.....	B <sub>1</sub>	99	100	100	101	123	103	99	99	101	96	110	102
2.....	B <sub>1</sub>	99	113	103	100	102	102	97	98	106	103	108	96
4.....	B <sub>1</sub>	101	96	91	100	102	104	99	103	107	107	98	94
5.....	B <sub>1</sub>	100	102	97	99	79	83	107	102	96	92	98	102
6.....	B <sub>1</sub>	99	94	100	101	88	82	111	99	91	96	91	98
7.....	B <sub>1</sub>	101	94	105	98	107	112	93	102	95	100	100	104
8.....	B <sub>1</sub>	100	102	104	102	98	113	93	98	104	105	98	102

When the seven constituents and four properties shown in Table 4 are considered together, it appears that the different samples of colloidal material are quite uniform in character. If a variation of 10 per cent from the mean is taken as "appreciable," it will be seen that only one of the colloids, 6-B<sub>1</sub>, differs appreciably from the mean in 3 of the 11 characteristics tabulated. Most of the colloids vary

appreciably in only 1 constituent or property. All appreciable variations, except one, occur in the  $\text{TiO}_2$ ,  $\text{MgO}$ , or  $\text{K}_2\text{O}$ , which together form only about 3 per cent of the whole colloid. Apparently, colloids taken from widely separated localities agree with one another as closely as colloids taken from practically adjacent spots. Samples 6 and 7, for instance, agree with each other or with samples 1, 2, and 3 about as closely as sample 1, 2, and 3 agree with one another; yet Table 1 shows that the localities from which samples 6 and 7 were taken are about 37 miles apart, whereas those of samples 1, 2, and 3 are only 5 and 200 yards apart.

In view of these data, it seems justifiable to conclude that the Leonardtown silt loam soil is characterized by a fairly definite kind of colloidal material. The typical Leonardtown colloid appears to be defined by its  $P_H$ , by its absorptive capacity for water, ammonia, and barium, and by its content of  $\text{SiO}_2$ ,  $\text{TiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{MgO}$ ,  $\text{K}_2\text{O}$ , and combined water. The percentages of organic matter,  $\text{SO}_3$ , and  $\text{P}_2\text{O}_5$  are too variable to be characteristic, and the contents of lime and manganese are definitive only in being low.

In connection with the conclusion that a type of colloid is characteristic of the Leonardtown type of soil, the nature of the soil sampling should be considered. As previously pointed out, the soil samples used in this work were not composites but were taken from single holes and embraced only a few cubic inches of soil. Since these small samples yielded uniform colloids, it is evident that with respect to colloidal material the Leonardtown type of soil is a fairly definite object even in small units.

Some idea of the number of samples that should be taken in order to determine the type of colloid that is characteristic of a soil type may be gained from the data given in Table 2. By use of the usual formulas for probable error,<sup>4</sup> the accuracy of the mean compositions given in the table and the limits within which a single sample should approach the mean may be estimated. It appears that in the case of the A-horizon colloids the chances are even that the means given in Table 2 agree with the true means within  $\pm 0.34$  per cent  $\text{SiO}_2$ ,  $\pm 0.20$  per cent  $\text{Fe}_2\text{O}_3$ ,  $\pm 0.28$  per cent  $\text{Al}_2\text{O}_3$ , and  $\pm 0.17$  per cent combined water, and there is an even chance that a single sample would agree with the mean within  $\pm 0.91$  per cent  $\text{SiO}_2$ ,  $\pm 0.53$  per cent  $\text{Fe}_2\text{O}_3$ ,  $\pm 0.72$  per cent  $\text{Al}_2\text{O}_3$ , and  $\pm 0.46$  per cent combined water. In the case of the B<sub>1</sub>-horizon colloids the probable errors of sampling are somewhat less, since these colloids are slightly more constant than the A-horizon colloids. Evidently, from 8 to 10 samples of a soil type would be sufficient to show the kind of colloid characteristic of the soil with a fair degree of surety.

Incidentally, the data obtained in this study are important in showing how closely different properties of the colloidal soil material may be expected to correlate with one another and with the chemical composition of the material. In previous studies of widely different colloidal soil materials, fair correlations were found between variations of the colloids in one property and variations in another; correlations were also apparent between variations in properties and

<sup>4</sup> The probable error of a single determination =  $0.6745\sigma$  and the probable error of the mean =  $\frac{0.6745\sigma}{\sqrt{N}}$ .

In these formulas  $\sigma$  represents the standard deviation and  $N$  the number of determinations.

variations in the  $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$  ratio (2, 8, 3). These relationships were established for a series of colloids in which wide variation occurred. In this series of colloids the variations are small; hence the relationships would probably appear less marked, the relationship not being absolute. It appears from Table 4, however, that in the case of these very similar samples of Leonardtown colloid there is some correlation between properties. The figures given in Table 3 for the adsorption of water vapor and ammonia gas show a fair correlation, the correlation coefficient between these two determinations being  $0.71 \pm 0.15$  for the A-horizon colloids and  $0.75 \pm 0.11$  for the B<sub>1</sub>-horizon colloids. The quantities of barium and water vapor adsorbed are less closely correlated, the coefficients for the A and B<sub>1</sub> colloids being  $0.66 \pm 0.17$  and  $0.58 \pm 0.17$ , respectively. There is no significant correlation between the adsorptions of barium and ammonia, and the adsorption data do not correlate with the comparatively constant  $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$  ratio. The slight correlation of properties in the case of these colloids of low variability is in harmony with the results of previous studies. It also serves to emphasize the fact that correlations between different properties and correlations between properties and chemical composition of the colloidal soil material are far from perfect.

#### DISCUSSION

The preceding data show that fairly typical spots of Leonardtown silt loam, selected at random, contain colloidal materials which are quite constant in their major constituents and adsorptive capacities. The nature of the colloidal material may therefore be considered a characteristic of this soil type. Further investigation is needed to show how constant other types of soil are in colloidal material; but it is fairly certain that the nature of the colloid may be considered a characteristic of many, if not all, types of soil.

Even if further investigation shows that each soil type is constant in its colloidal material, many data must be collected before it is evident just how valuable the composition of the colloid is as a classification characteristic. On the basis of the four major constituents

$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$  or of the ratio, only a few groups of soil types could be distinguished; but some of the more variable minor constituents will doubtless distinguish additional subordinate groups. It is not unreasonable to expect that the kind of colloidal material will prove as valuable a characteristic for soil classification as the quantity of clay or colloid has proved to be.

Aside from any value in soil classification, a knowledge of the kinds of colloidal material in the different cultivated areas is important for throwing light on the properties and management of soils; and it appears from this study that such knowledge may be approximated by analyzing the colloidal materials present in a comparatively few samples typical of each type of soil. The probable error calculations given on page 467 show that in the case of the Leonardtown silt loam, seven samples of soil taken with only reasonable care showed

the kind of colloid typical of this soil type with considerable accuracy, and that samples of colloid from individual spots do not show a wide variability in their major constituents. Of course, it does not follow from these data that all areas that have been mapped as Leonardtown silt loam will contain colloidal materials as similar to the mean as the typical samples that have been analyzed. But the colloid of any particular area should at least be similar to the mean of the type in proportion as it is typical Leonardtown, since typical spots are fairly constant.

The data given in Table 2 throw light on two questions that are not immediately related to this study. It was pointed out that the colloidal material from the A horizon has very nearly the same composition as that of the B<sub>1</sub> horizon when the results are expressed on an inorganic basis. This is in agreement with the results of a previous investigation in the course of which analyses were made of colloids isolated from the A and B horizons of 15 types of soil (18). In each case the A horizon colloid was very similar to the B colloid in inorganic composition. It thus seems that the colloids in these two horizons are similar in most types of soil. Results recently obtained by McCool on some Michigan soils indicate, however, that this is not universally true (14). The fact that colloidal material in the B<sub>2</sub> horizon is also very similar to that in the A and B<sub>1</sub> horizons is somewhat surprising, since the B<sub>2</sub> horizon is a distinct hardpan. Data given in Table 1 shows that this indurated layer contains considerably less colloidal material than the friable B<sub>1</sub> horizon; hence it would appear that the hardness characteristic of this layer is due neither to the kind nor to a particular quantity of colloidal material present. Possibly it is primarily dependent on a peculiar structure of the non-colloidal material.

#### SUMMARY

This study was conducted for the purpose of determining the variability in the colloidal material of typical samples of Leonardtown silt loam soil. The data reported concern the chemical composition and properties of the colloidal materials that were isolated from random samples typical of this silt loam.

The colloids of this type obtained from different localities are, on the whole, fairly constant in hydrogen-ion concentration, in adsorptive capacity for water vapor, ammonia gas, and barium, and in content of SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, combined water, TiO<sub>2</sub>, K<sub>2</sub>O, and MgO. The MnO and CaO are more variable, but these constituents are low in all samples. Organic matter, SO<sub>3</sub>, and P<sub>2</sub>O<sub>5</sub> vary widely. It thus appears that the Leonardtown silt loam type of soil is characterized by a type of colloid which is fairly definite in adsorptive properties and in those inorganic constituents that are present in appreciable quantities.

The probable errors of sampling indicate that 8 to 10 samples of a type of soil should be sufficient to show with considerable accuracy the kind of colloidal material characteristic of the type.

Incidentally, the data obtained in this study confirm a conclusion of previous work, that in most soils the colloidal materials of the A and B<sub>1</sub> horizons are very similar in inorganic composition. The data also indicate that the hardness of the B<sub>2</sub> horizon in this type of soil is not primarily dependent upon the kind or quantity of colloidal material present.

## LITERATURE CITED

- (1) ANDERSON, M. S.  
1924. THE HEAT OF WETTING OF SOIL COLLOIDS. *Jour. Agr. Research* 28: 927-935.
- (2) ——— and MATTSON, S. E.  
1925. THE RELATION BETWEEN PROPERTIES AND CHEMICAL COMPOSITION OF SOIL COLLOIDS. *Science* (n. s.) 62: 114-115.
- (3) ——— and MATTSON, S. [E].  
1926. PROPERTIES OF THE COLLOIDAL SOIL MATERIAL. U. S. Dept. Agr. Bul. 1452, 47 p., illus.
- (4) ——— FRY, W. H., GILE, P. L., MIDDLETON, H. E., and ROBINSON, W. O.  
1922. ABSORPTION BY COLLOIDAL AND NONCOLLOIDAL SOIL CONSTITUENTS. U. S. Dept. Agr. Bul. 1122, 20 p.
- (5) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (6) BRADFIELD, R.  
1925. THE CHEMICAL NATURE OF COLLOIDAL CLAY. *Jour. Amer. Soc. Agron.* 17: 253-270, illus.
- (7) DAVENPORT, E.  
1907. PRINCIPLES OF BREEDING. 727 p., illus. Boston, New York [etc.].
- (8) GILE, P. L.  
[1925.] NATURE OF THE COLLOIDAL SOIL MATERIAL. In Holmes, H. N., and Weiser, H. B., editors, *Colloid Symposium Monograph* [v. 3], p. 216-227, illus. New York.
- (9) ———  
1927. CHEMICAL DETERMINATIONS TO BE MADE IN THE COURSE OF A SOIL SURVEY. *Jour. Amer. Soc. Agron.* 19: 285-289.
- (10) ——— MIDDLETON, H. E., ROBINSON, W. O., FRY, W. H., and ANDERSON, M. S.  
1924. ESTIMATION OF COLLOIDAL MATERIAL IN SOILS BY ADSORPTION. U. S. Dept. Agr. Bul. 1193, 42 p.
- (11) GILLESPIE, L. J.  
1916. THE REACTION OF SOIL AND MEASUREMENTS OF HYDROGEN-ION CONCENTRATION. *Jour. Wash. Acad. Sci.* 6: 7-16, illus.
- (12) HALL, A. D., and RUSSELL, E. J.  
1911. SOIL SURVEYS AND SOIL ANALYSES. *Jour. Agr. Sci. [England]* 4: [182]-223.
- (13) HILLEBRAND, W. F.  
1919. THE ANALYSIS OF SILICATE AND CARBONATE ROCKS. U. S. Geol. Survey Bul. 700, 285 p., illus.
- (14) MCCOOL, M. M.  
1927. RESULTS OF SOME PHYSICAL AND CHEMICAL STUDIES ON SOIL COLLOIDS. *Jour. Amer. Soc. Agron.* 19: 289-297.
- (15) MIDDLETON, H. E.  
1924. FACTORS INFLUENCING THE BINDING POWER OF SOIL COLLOIDS. *Jour. Agr. Research* 28: 499-513, illus.
- (16) ROBINSON, G. W.  
1917. STUDIES ON THE PALÆOZOIC SOILS OF NORTH WALES. *Jour. Agr. Sci. [England]* 8: [338]-384, illus.
- (17) ROBINSON, W. O.  
1922. THE ABSORPTION OF WATER BY SOIL COLLOIDS. *Jour. Phys. Chem.* 26: [647]-653.
- (18) ——— and HOLMES R. S.  
1924. THE CHEMICAL COMPOSITION OF SOIL COLLOIDS. U. S. Dept. Agr. Bul. 1311, 42 p.

# A CHEMICAL STUDY OF THE DEVELOPMENT OF COTTON BOLLS AND THE RATE OF FORMATION OF GOSSYPOL IN THE COTTON SEED.<sup>1</sup>

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## INTRODUCTION

The significance of small amounts of gossypol and its related compounds in cottonseed products has been emphasized in previous communications (6, 7).<sup>3</sup> In a series of investigations to determine the factors which affect the gossypol content of cottonseed meal it became necessary to study the rate of formation of gossypol in the cotton seed and to determine the stage of development of the seed at which gossypol was present in the greatest amounts. The quantity of this toxic substance in the meal is largely dependent upon the quantity in the original seeds, and this in turn is probably governed somewhat by the nutrition of the plant. Evidence of the existence of such a dependent relationship has been previously reported (15), and a positive correlation found to exist between the gossypol content of the seed and its oil content. The practical application of such findings, however important, has not received a great deal of attention.

In a previous investigation (7) it was observed that gossypol began to form in the cotton seed previous to the opening of the boll and reached its maximum amount soon after the boll had opened. The oil developed in the seed in increasing amounts at the same time, although not in the same proportion. The age of the seeds was calculated from the stage of development of the boll.

## EXPERIMENTAL

In the study reported here, which had for its purpose the further investigation of the apparent relationship existing between the formation of gossypol and of oil during the development of the seed, more rigid measures were taken to determine the exact age of the experimental material and reduce variability in samples to a minimum. To reach this objective and make the results comparable to those obtained a year previous and recently reported (7), the same variety of cotton seeds was used and the seeds were obtained from plants growing in the same area of the field. When the plants were in bloom the flowers were marked with the date at which they were fully open, and this date was used to determine the age of the bolls which were later collected. A sufficient number of flowers were

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 480.

marked during 10 days of the flowering period to insure a large quantity of representative samples. Eight days after the first flowers were marked a collection of bolls was made and further collections were made at the intervals shown in the tables which follow. By this procedure a considerable amount of experimental material other than the seeds which were needed for the gossypol determinations was collected, and it appeared worth while to include in the study the analysis of the several parts of these cotton bolls as they matured.

Although an enormous amount of work has been done on the chemical composition of the cotton plant as a whole, little attention has been given to the composition of the contents of the boll in the various stages of development, and it seemed for several reasons that this might prove of some importance. The need for specific information of this sort is felt most in finding new uses for waste products which accumulate each year. In fact, the data collected during this part of the investigation yielded such interesting results that they tended to subordinate the primary object of the study, which was mainly concerned with the formation of gossypol and oil in the cotton seed.

Unopen or partly open bolls which fail to mature and are left in the field have practically no value at the present time, although in some years it has been a practice at the close of the cotton-picking season to collect these bolls and sell them to the ginners. Bolly refuse, consisting mainly of the burr and some unginned cotton and which collects at the cotton gins in large quantities, has been suggested as a feed of some value (5). The ash of this material is high in fertilizing constituents. From the results presented in this paper the composition of cotton lint itself might be expected to show some variation from year to year, a fact which would impair its value for certain specialized purposes. Brown (3) quite correctly states that "cotton lint is often spoken of as being pure cellulose, but \* \* \* it contains several other materials in considerable quantity."

For the sake of brevity and to facilitate the comparison of a large number of analyses, the results of this study are presented in several tables with a discussion following each. The proximate analyses were made by the methods of the Association of Official Agricultural Chemists (1) and the gossypol determined by Carruth's method as modified by Schwartze and Alsberg (15).

#### DISTRIBUTION OF MATERIAL IN COTTON BOLLS

The weight of the dry material obtained from 25 bolls of cotton at different stages of maturity is shown in Table 1. In the data here presented a small increment of error may have been introduced through the use of the calculated, rather than the actual, weight of some of the materials. It is unfortunate for the completeness of this table that the cotton burrs were not preserved during the first part of the experiment, but as their analyses would be of little value to the main object of the study, the calculation of these values seemed justifiable.



TABLE 1.—Weight of dry material obtained from 25 bolls of cotton at different stages of maturity

Age of material	Size of bolls	Weight of—				
		Bolls	Contents of bolls	Lint	Seeds	Burrs
	Cm.	Gm.	Gm.	Gm.	Gm.	Gm.
8 days.....	2.5	25.16				
16 days.....	3.4	84.25	46.86	4.88	30.35 ° (870)	° 37.39
24 days.....	3.5	121.65	62.19	13.40	31.52 ° (877)	° 59.46
32 days.....	3.5	136.84	109.90	41.56	51.28 ° (725)	° 28.94
46 days.....	3.5	212.73	171.20	56.50	94.85 ° (862)	41.53
52 days.....		214.89	169.60	60.75	117.87 ° (1,047)	45.29

° Number of seeds in 25 bolls.

° This figure represents the calculated rather than the actual weight.

It may be noticed that the combined weights of the dry seeds and lint of 25 bolls do not agree with the dry weights of the boll contents, and that after 24 days this percentage difference becomes less at each successive stage of maturity. These differences are taken as representing water-soluble material removed from the seeds and lint during the preparation of the samples, as described later. Moreover, the figures given are the averages obtained from a large number of samples calculated on the basis of 25 bolls, and not the weights of material obtained from a few selected samples, which would no doubt give a distorted picture of actual conditions, due to a surprisingly large variation in size of bolls.

The separation of the lint from the seeds was done by hand and complete separation was easily accomplished in those instances in which the material was very wet. As the seeds became more mature the lint was more firmly attached and its final removal was effected with sulphuric acid. By determining the dry weight of a given number of seeds before and after delinting, the residual lint, more properly termed "fuzz" or "linters," was found and the proper correction made for it in determining the total amount of lint in the bolls.

The number of seeds in 25 bolls, which is given in parenthesis to the right of the weight of the seeds, shows some variations that must be taken into account in the analysis given later. These variations, which undoubtedly contribute an element of error when the percentage composition of the material produced by the plant is used as an index of quality without taking into consideration the quantity produced, was controlled as much as possible by discarding the bolls showing insect injury or abnormal conditions. Fortunately, very few bolls at any of the stages given were unsuitable as experimental material. The bolls 32 days old yielded the smallest number of seeds and those 52 days old the largest number, while the others were quite uniform in respect to number present. The mature bolls should contain no more seeds than the immature ones, although this is only true providing the development of some of the seeds has not been prevented by some injury which might have passed unnoticed in the immature bolls but which was sufficiently apparent in the mature boll to mark it as unsuitable for analysis. With these explanatory remarks, and excepting the number of seeds found in the bolls 32 days old, the author believes that Table 1 represents the average distribution of material in healthy cotton bolls from plants of Oklahoma 44 variety grown under normal conditions.

## CHEMICAL ANALYSES

The results of the analyses of the cotton bolls and their contents are given in Table 2.

TABLE 2.—*Chemical composition of cotton bolls and contents at different stages of maturity, determined on a dry-matter basis*

BOLLS					
Age of material	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
8 days.....	4.16	13.91	7.03	70.68	4.22
16 days.....	4.10	14.24	16.50	62.32	2.84
24 days.....	3.80	12.44	24.86	56.09	2.81
32 days.....	3.57	11.81	40.20	37.00	7.42
46 days.....	3.81	13.72	46.89	22.94	12.64
52 days.....	3.93	12.75	51.27	21.28	10.77
BOLL CONTENTS					
16 days.....	4.28	13.96	12.62	65.92	3.22
24 days.....	3.36	12.01	27.50	53.52	3.61
32 days.....	2.98	13.36	46.27	28.20	9.10
46 days.....	2.55	15.50	47.00	19.81	15.14
52 days.....	2.50	14.60	53.23	16.65	13.02
LINT					
16 days.....	2.85	10.63	46.57	35.11	4.84
24 days.....	1.03	4.75	72.68	18.80	2.74
32 days.....	.62	1.67	88.98	8.19	.54
46 days.....	.34	.23	89.66	9.49	.28
52 days.....	.42	.42	93.34	5.53	.29
SEEDS					
16 days.....	3.15	16.04	8.97	70.84	1.00
24 days.....	3.75	17.27	19.56	56.30	3.12
32 days.....	3.76	22.89	19.56	31.44	22.35
46 days.....	3.48	26.95	18.20	26.25	25.12
52 days.....	3.53	26.96	19.12	25.42	24.97

## BOLLS

In the previous discussion, attention was called to the manner in which the age of the bolls was determined by dating the flowers and counting the number of days from that date until the bolls were picked. The cotton flowers usually wither and fall off the second day after they open and the boll which develops from the enlarged ovary of the flower rapidly grows to full size. After each picking the bolls were brought to the laboratory and weighed. They were then dried at 100° C. for one hour or slightly longer, and finally air dried. Analysis was made immediately on the ground air-dry material and the results computed to the dry-matter basis. The bolls eight days old were so small and undeveloped that a separation of their parts for analysis was not attempted.

As the bolls matured the percentage of ash and crude protein remained quite constant, nitrogen-free extract decreased rapidly, and crude fiber and ether extract both increased. None of the bolls

had opened until after the fortieth day and in the collection made on the forty-sixth day only 10 in 100 had opened. The composition of these unopened bolls was much the same as the composition of mature bolls which were entirely open and picked six days later. Although this similarity in composition is not startling, it is of some consequence and worthy of attention. Dowell and Friedemann (5) made analyses of the seeds obtained from bollies (the term applied at the cotton gin to unopen bolls) and compared the properties of the oil obtained from such seed with those of the prime crude oil. They were unable to draw definite conclusions as to the value of the bolly oil and were of the opinion that the composition of the seeds would vary from year to year. From the data they presented one might expect the oil to be of inferior quality. Other investigators have reported the analysis of cotton bolls at different stages of development, but for the most part these analyses have been made on material of which the age was only approximate, or else certain assumptions were made which rendered the results of comparative interest only, although suitable for the purpose of the particular investigation. The earliest of these investigations are reported in Bulletin No. 33, United States Department of Agriculture, Office of Experiment Stations (16).

#### BOLL CONTENTS

The contents from a selected number of bolls were handled as described above and care was exercised to retain in the boll contents all the material which belonged to them. In this material the percentage of ash showed a decrease as the boll matured and as the lint, which is low in ash, developed. The protein remained quite constant since nitrogen is present in relatively large amounts at an early stage and increases in the seeds as it decreases in the lint during the period of maturation. Both crude fiber and ether extract increased rapidly. Further comment on these results is not necessary.

#### LINT

The composition of cotton lint, which is of little importance from the standpoint of the demands made upon the fertilizing constituents of the soil during the growth of the plant, is of considerable importance in relation to the utilization of the product. As previously pointed out, and contrary to the opinion of many not directly connected with the problems involved in textile work, the lint contains appreciable amounts of material other than cellulose. The presence of these materials, sometimes referred to as natural impurities, were recognized as early as the middle of the nineteenth century, although it was not until comparatively recent years that any great significance was attached to them. The only other study made upon the composition of cotton lint during successive stages of development, with which the writer is familiar, is one reported by Levine (10) several years ago. The study mentioned was carried out to elucidate results of a previous investigation by Hebden (9), who was probably the first to point out the possibility that the proteins of the fiber play an important part in the bleaching of cotton cloth. Levine determined the protein content and the content of ether and alcohol-

soluble material in the fibers at two-day intervals from the time the boll was about 16 days old until it was 38 days old. Although this investigator did not specify the manner in which the experimental material was handled nor the basis on which his calculations were made, the results were of such importance that he pointed out the necessity of careful investigation of the nature of the fatty and waxy substances and a study of the effect of growth on these constituents of the cotton fiber. It is in connection with this latter aspect of the problem, as well as the protein content of the fibers, that certain studies have been promoted at this station.

TABLE 3.—*Chemical composition of cotton lint as reported by different investigators*

Approximate age of lint	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Analyst
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
16 days.....		13.94				Levine.
26 days.....		2.35			1.74	Do.
32 days.....		1.66			1.52	Do.
38 days.....		1.13			1.41	Do.
52 days.....	0.42	.42	93.34	5.53	.29	Gallup.
	1.77	1.61	89.75	6.22	.65	McBryde (11).
	1.25	1.12	87.02	10.00	.61	Ross (14).

In Table 3 are reproduced the results of several analyses of cotton lint reported by different investigators. These figures are themselves sufficient evidence for assuming the composition of the lint to be variable and worthy of further study. The figures presented in Table 2, which represent the composition of the lint at different stages of maturity, were obtained by analyzing samples washed free of adhering material. When the bolls were collected, the lint was immediately separated from the seeds and washed several times with cold water. Each sample was treated alike, and although such a procedure is subject to some criticism, it no doubt affords the best means of obtaining representative samples, which would be impossible if the total contents of the boll without the seeds were taken as representing lint.

#### SEEDS

Many analyses of mature cotton seeds have been made by various investigators, but little attention has been given to the rate of formation and development of the oil in the seed. The manufacturer of cottonseed oil is primarily interested in the amount of oil in the seed, and, although its protein content is of secondary importance, in late years there has been a growing endeavor on the part of the purchaser to obtain seeds high in both. This increasing demand for seeds of high oil content has prompted plant breeders and others to attempt to increase the oil and protein content of seeds by proper selection and various fertilizer treatments. The work of Garner, Allard, and Foubert (8), Wells and Smith (17), Creswell and Bidwell (4), and in particular Rast (13)—who concludes that “by eliminating the inferior varieties the quality of the seed could easily be improved, thereby increasing their average value \$5 per ton”—

has gone far toward promoting studies along this line. Many others have contributed information of equal importance.

An inspection of the figures in Table 2, showing the rate of formation of oil in the seeds, leads one to believe that there is a period of intense oil formation occurring in the seeds 32 days old. This might at first appear to be at variance with what has been previously reported and is reproduced with the later results in Table 4. In the year 1926, when a similar study was made, the exact age of the seeds analyzed was not known, and since the size of the boll would be of no value in determining this, its condition with respect to opening was taken as the most logical basis for determining the stage of development of the seed. For that reason the results do not lend themselves to direct comparison with those obtained in this later study unless certain reservations are made. Again referring to the figures in Table 2, with special reference to the changes in oil and protein, it may be pointed out that the increase in protein as the seeds develop is much less than that in oil and shows no critical period of formation. The increase of both is relatively slight during the last 20 days. From this it might be reasoned that there is a deposition of nitrogen early in the life of the seed, and that its increase, though fairly constant, is proportionally greater than that of the growth of the seed. In contrast to this, the oil developed at a well-defined stage of maturity without showing any relation to the growth of the seed before or after this critical period. Certainly some one or more factors other than the actual age of the seed must have been responsible for the low percentage of oil recovered by Dowell and Friedemann (5) in their studies with bolly refuse and by Garner, Allard, and Foubert (8) in their work with seeds from immature and mature bolls. Perhaps these differences may be explained on the assumption that the composition of the seed is influenced by seasonal variations, and for this there is some experimental evidence. Martin, Ballard, and Simpson (12) found that the period of maturation of the seeds increased as the season advanced. In another investigation (8) it was concluded that climate was a potent factor in determining conditions favorable to formation of oil. Other studies (2) have shown a close relation between the amount of rainfall during the cotton-growing season and the amount of oil in the seeds. Since the bolls are formed on the plants at different times, the earliest ones being found toward the bottom of the plant and close to the main stem, it is quite obvious that the period of oil formation occurring in the late-developing seeds may be subject to vastly different and often unfavorable growing conditions. A systematic study of the development of oil, or any other constituent in the seed, with the hope of increasing the amount of any one of them would therefore require special precautions whereby all variables could be controlled and kept fairly constant. These points, which should be borne in mind when interpreting the experimental data presented in Table 2, have special significance in attempting a comparison of results such as has been made in Table 4.

TABLE 4.—*Development of oil and gossypol in cotton seeds*

## AS PREVIOUSLY REPORTED

Description of material	Oil in seeds	Gossypol in seeds
	<i>Per cent</i>	<i>Per cent</i>
Unopen bolls.....	13.97	0.48
Open bolls.....	24.01	.428
Bolls open over six days.....	24.41	.538

## PRESENT INVESTIGATION

Bolls 16 days old.....	1.00	-----
Bolls 24 days old.....	3.12	-----
Bolls 32 days old.....	22.35	0.230
Bolls 46 days old.....	25.12	.634
Bolls 52 days old.....	24.97	.538

## AVERAGES REPORTED BY OTHER INVESTIGATORS

Bollies <sup>a</sup> .....	12.20	( <sup>b</sup> )
Immature bolls <sup>c</sup> .....	20.4	( <sup>b</sup> )
Mature bolls.....	23.5	( <sup>b</sup> )

<sup>a</sup> Dowell and Friedemann (5). With reference to the oil content of the seeds from bollies, the investigators state that no definite conclusions can be drawn as the composition of the seeds will probably vary from year to year. No doubt these seeds, which were obtained from various oil mills, represented a more heterogeneous sample than any of the other ones represented.

<sup>b</sup> Not determined.

<sup>c</sup> Garner, Allard, and Foubart (3). These investigators state that "immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots." From this it is presumed that the immature bolls were about 40 days old.

## FORMATION OF GOSSYPOL

The seeds on which the gossypol determinations were made after removing the lint were dried at a sufficiently low temperature to prevent the destruction of gossypol. Otherwise they were handled in the same manner as the other materials. From the figures presented in Table 4, it is quite evident that the formation of gossypol was concurrent with that of oil, and from these figures the idea might be gained that some relation existed between the two. In view of the importance of such a relation, in considering the function of gossypol, the experimental basis for such a view calls for careful consideration and far more data than have been obtained up to the present time. Too little is known about gossypol to even postulate that it might act as an accelerator for the formation of oil, although such a conception is no more far-reaching than many others which have been made and later proved. Gossypol could not be detected in seeds from bolls less than 32 days old and no doubt the ether-extracted material of the seeds previous to this time was made up in part of soluble material other than oil. However, there still remains the possibility of gossypol being present in these younger seeds in a form which is not ether soluble and does not respond to the usual tests for gossypol.

It was observed during the extraction with ether of the fat-soluble material from the seeds that the color of the first portion of the extract which ran through was red only in the case of the more mature seeds. The seeds from bolls 16 and 24 days old gave no such color to the extract and contained very little oil. Although this red color is characteristic of solutions of gossypol, other mate-

rials of a questionable nature and present largely in the seed coating give a similar color, and it is probable that all of these made their appearance at about the same time. The absence of red very likely indicates the absence of even small traces of gossypol, but this should not be taken as conclusive proof.

It should be kept in mind that the age of the seeds whose oil and gossypol content are shown in the first part of Table 4, was approximated by observing the condition of the boll; a procedure which, as previously indicated, may not be entirely reliable. It would be hardly possible to discriminate by appearance alone between bolls 24 and 32 days old, and even when the first signs of boll opening are taken as representing material of about the same age, one is confronted with the possibility of short maturation periods with subsequent variations in the final composition of the seeds. Such an explanation may account for the small amount of gossypol and relatively large amount of oil found in the seeds from bolls designated as unopen. What has been said in this respect will also apply to the oil content of seeds from bollies.

Schwartz and Alsberg (15) made the important observation that the variation in the gossypol content of seeds tended to vary directly with and bore a true relationship to the oil content. Seeds from the Southwest tended to be low in oil and gossypol, those from the Southeast to be somewhat higher, and those from the Pacific coast regions to be still higher. This again points to the influence of climatic conditions on the development of the oil and the above relationship is unfavorable from the viewpoint of producing seeds high in oil and low in gossypol. If the latter situation could be met it would be of value only in so far as it could be accomplished without detriment to the other ingredients in the seed. Such an achievement would indeed be a valuable contribution to the cottonseed oil industry and all others concerned with its products.

#### SUMMARY

A study of the change in composition of cotton bolls picked at different stages of development showed that the changes were very rapid from the time the boll first formed until it was ready to open. The young bolls were high in ash, nitrogen, and carbohydrates, and as they matured the fat and crude fiber increased. After the bolls had reached the opening stage their composition was altered slightly by an increase in crude fiber, but from the results presented, the nearly mature but unopen bolls will yield products almost as high in their several constituents as the open ones. The composition of bollies and bolly refuse which are composed of immature bolls and their parts in various stages of development, seems to depend upon a number of factors, the most important of which is probably the age of the boll, followed by time of maturation, climatic conditions during growth, and possibly the nutrition of the plant.

Cotton lint is quite variable in composition and although the mature lint is composed mostly of crude fiber, there are other materials present in sufficiently large amounts to be of questionable importance.

The development of oil and gossypol in the cotton seed occurred at about the same time and during a very short and well-defined period

of growth. Although it is too early to predict the function of gossypol in the development of the seed, it seems to be associated in some way with the formation of oil. A careful study of all the factors which may influence the growth of the seed, together with an endeavor to increase the oil and decrease the gossypol, is needed.

#### LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (2) BARROW-AGEE LABORATORIES, INC.  
1918. RAINFALL AND OIL CONTENT [IN COTTONSEED]. Cotton Oil Press 2 (8): 44-45, illus.
- (3) BROWN, H. B.  
1927. COTTON. 517 p., illus. New York and London.
- (4) CRESWELL, C. F., and BIDWELL, G. L.  
1921. COMPOSITION OF COTTONSEED. U. S. Dept. Agr. Bul. 948, 221 p., illus.
- (5) DOWELL, C. T., and FRIEDEMANN, W. G.  
1918. EXPERIMENTS WITH BOLLY REFUSE. Okla. Agr. Expt. Sta. Bul. 121, 8 p.
- (6) GALLUP, W. D.  
1926. ELIMINATING THE TOXICITY OF COTTONSEED MEAL. Jour. Dairy Sci. 9: 359-372.
- (7) ———  
1927. THE GOSSYPOL CONTENT AND CHEMICAL COMPOSITION OF COTTONSEEDS DURING CERTAIN PERIODS OF DEVELOPMENT. Jour. Agr. Research 34: 987-992.
- (8) GARNER, W. W., ALLARD, H. A., and FOUBERT, C. L.  
1914. OIL CONTENT OF SEEDS AS AFFECTED BY THE NUTRITION OF THE PLANT. Jour. Agr. Research 3: 227-249.
- (9) HEBDEN, J. C.  
1914. CHEMISTRY OF THE BLEACHING OF COTTON CLOTH. Jour. Indus. and Engin. Chem. 6: 714-720.
- (10) LEVINE, B. S.  
1915. THE IMPORTANCE OF A CONSIDERATION OF THE FIBER PROTEINS IN THE PROCESS OF BLEACHING COTTON. Science (n. s.) 41: 543-545.
- (11) MCBRYDE, J. B.  
1891. A CHEMICAL STUDY OF THE COTTON PLANT. Tenn. Agr. Expt. Sta. Bul. 4 (5): [120]-145.
- (12) MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M.  
1923. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 195-208, illus.
- (13) RAST, L. E.  
1917. THE OIL CONTENT OF COTTONSEED AN ACCURATE BASIS FOR COMMERCIAL STANDARDIZATION. Science (n. s.) 45: 507-508.
- (14) ROSS, B. B.  
1899. THE COTTON PLANT CONSIDERED IN SOME OF ITS CHEMICAL RELATIONS. Ala. Agr. Expt. Sta. Bul. 107: 369-402.
- (15) SCHWARTZ, E. W., and ALSBERG, C. L.  
1923. QUANTITATIVE VARIATION OF GOSSYPOL AND ITS RELATION TO THE OIL CONTENT OF COTTONSEED. Jour. Agr. Research 25: 285-295, illus.
- (16) UNITED STATES DEPARTMENT OF AGRICULTURE, OFFICE OF EXPERIMENT STATIONS.  
1896. THE COTTON PLANT. U. S. Dept. Agr., Off. Expt. Stas. Bul. 33, 433 p., illus.
- (17) WELLS, C. A., and SMITH, F. H.  
1915. THE NITROGEN AND FAT IN SHORT STAPLE COTTONSEED. Jour. Indus. and Engin. Chem. 7: 217.



## AN OBSERVED CASE OF "SPONTANEOUS" IGNITION IN STABLE MANURE.<sup>1</sup>

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Instances of "spontaneous" ignition of farm products which have occurred under actual observation are sufficiently rare to warrant the publication of all such available data. Studies of the agents active in "spontaneous" heat production have been conducted by the senior author for several years, but it was only by good fortune that the present writers were enabled to study a case of fire at first hand.

A number of authentic detailed accounts of "spontaneous" ignition in farm products have been reported in the past decade.<sup>2</sup> That fires in such materials do occur from heat generated within the mass can be doubted no longer, though some observers still attribute the conflagration to sparks, carelessly discarded matches, etc.

The observations reported in this paper were made upon a large pile of stable manure and straw at the Arlington Experiment Farm, Rosslyn, Va. Manure for fertilizer purposes had been hauled from a neighboring cavalry station and placed on the farm grounds in an open plot. As the manure was unloaded the horses and wagons were driven over the pile, and the load was deposited on the top. The custom had been to "cure" the manure from one to three years before spreading it. The first loads had been deposited some two and one-half years before, and the mass had grown to a pile about 200 feet long, 50 feet wide, and from 1 to 20 feet high. (Fig. 1.) Moderate heating of the manure had been accepted as an essential part of the curing, though fire had not formerly occurred.

Before September 9, 1925, the date of the outbreak, the weather had been warm, ranging from 87° to 95° F. There had been no heavy rains for two weeks. Daily additions had been made to the pile, and, although the mass was known to be excessively hot, the condition was not considered dangerous. Fire broke out during the night of September 9, and was discovered by the night watchman. The flames quickly spread over the entire west side of the long pile. Water was applied from pails, and the flames were extinguished. Within a few hours, fire again broke out along the same side of the mass. When this was controlled, efforts were made to cut away as much of the material as was still at a dangerously high temperature. Charred straw and manure to a depth of about 2 feet throughout the greater part of the length of the pile were removed. The material was firmly packed, black, dry, and brittle to a depth considerably beyond any possible penetration by the fire. (Fig. 2.)

<sup>1</sup> Received for publication Dec. 24, 1927; issued May, 1928.

<sup>2</sup> JAMES, L. H. STUDIES IN MICROBIAL THERMOGENESIS. I. APPARATUS. *Science* (n. s.) 65: 504-506, illus. 1927.

RETTGER, L. F., and THOM, C. MICROBIAL THERMOGENESIS. II. HEAT PRODUCTION IN MOIST ORGANIC MATERIALS WITH SPECIAL REFERENCE TO THE PART PLAYED BY MICROORGANISMS. *Jour. Bact.* 15: 117-141. 1928.

The temperatures of various points in the mass were determined with the aid of a thermocouple and potentiometer. Holes were made by forcing a long iron rod into a pile; the thermocouple was



FIG. 1.—Pile of stable manure, showing area of third outbreak of fire; east side

inserted in the openings. A series of readings (Table 1) indicated that in the center of the pile and up to within 5 or 6 feet of the outer surface the temperatures were not extremely high, averaging only

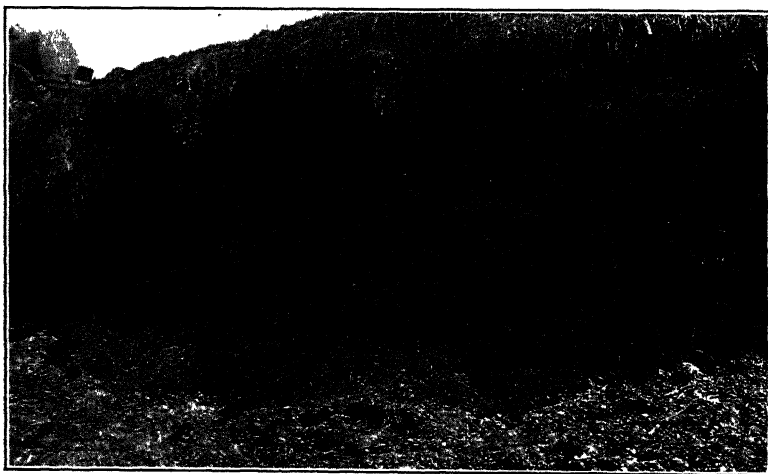


FIG. 2.—Site of first and second outbreaks of fire, showing compact character of manure pile; west side

about 51° C.; 66° was the maximum. The temperatures of the outer layers, however, were considerably higher. In places where the manure was firmly packed, as on the dismantled side, the highest temperature was usually within 6 inches of the surface, whereas on the top, where recent additions still lay more or less loosely, the maximum temperature was usually found from 1½ to 2 feet within the mass. •

TABLE 1.—*Temperatures obtained with a thermocouple in different areas of a large pile of stable manure*

Reading	Surface	Depth	Temperature, °C.
1	Top.....	12 inches.....	50.
2	do.....	do.....	66.
3	do.....	48 inches.....	36.
4	Side.....	12 inches.....	67.
5	do.....	6 inches.....	77.
6	do.....	do.....	82.
7	do.....	do.....	80.
8	do.....	12 inches.....	78.
9	do.....	6 inches.....	76 near smoking straw.
10	do.....	Surface.....	87 in smoking straw.
11	do.....	do.....	132 in glowing straw.
12	do.....	Removed from pile.....	167 in glowing straw.

The temperature of the opposite or east side of the pile was also excessively high, though no fire had yet appeared. This side was



FIG. 3.—Site of third outbreak of fire; A, location of hottest belt; east side

watched carefully. The following afternoon, September 10, fire broke out on this side, appearing first, as the others had, at from 5 to 10 feet above the ground.

When this had been put out an inspection of the stack revealed so many hot areas along the edge that the entire side was removed under frequent sprinkling with water. (Fig. 3.)

The mass was packed so firmly that progress was slow and fire broke out twice before the work was completed. In one of the hottest sections (fig. 3, A) a forkful of material was removed and spread out upon the ground. The straw was hot and steaming. About one minute later the steam had changed to smoke, which increased in density until after about three minutes the material glowed a fiery red. Exactly the same conditions appeared in the stack adjacent to the spot from which the material had been removed.

Temperature readings of the glowing coals and adjacent materials (though those of the former are probably only approximate) gave the following results:

	° C.
Temperature of glowing straw removed from pile.....	167
Temperature of glowing straw remaining in pile.....	132
Temperature in stack 3 inches from glowing coals.....	80

The fire smoldered a few minutes, then went out. It is significant that the temperature of material only a few inches from the glowing coals was not above 80° C. Moisture determinations showed that material in the stack adjacent to the point where the red glow had appeared contained only 3.7 per cent of water, whereas material 2



FIG. 4.—Normal and charred material

feet away, apparently just as badly scorched and brittle, contained 30.2 per cent. Material from the top of the stack, which was hot but showed no signs of scorching, contained 66 per cent moisture.

Figure 4 shows the contrast between material which has undergone excessive heating without firing and normal material from the surface of the pile. The charred material was very dry and brittle.

As the heating mass of material offered excellent opportunities for study, the following day further data were obtained. In general, the mass had cooled considerably, though a few areas were still hot.

Since "spontaneous" ignition has been known to take place apparently only as the result of rapid oxidation, it was assumed that further temperature rise would be produced in the manure pile on the introduction of air, or, preferably, pure oxygen. A brass tube and the thermocouple were introduced simultaneously into the center of an area having a temperature of 82.5° C. At 11.25 a. m. oxygen was introduced from a pressure tank, and changes in temperature were carefully noted. The temperature immediately began to rise rapidly and continued to increase for one-half hour; the rate of oxygen flow was occasionally increased. (Table 2.) At 12 m. the temperature had reached 109° C., an increase of 26.5° in 30 minutes. Further aeration resulted in a slow cooling to 107° at 1 p. m. As the heating

material was inclosed within the mass, the estimation of the oxygen requirements was purely conjectural. Thus it is not surprising if the oxygen rate most favorable for heat production was not maintained and only limited heating allowed to take place.

TABLE 2.—*Temperatures obtained by introducing oxygen into hot area of manure pile (2 feet beneath side surface)*

Reading	Time	Temperature	Oxygen flow	Reading	Time	Temperature	Oxygen flow
		° C.				° C.	
1	11.24 a. m.-----	82.5	Begun.	8	11.43 a. m.-----	106.0	Increased.
2	11.25 a. m.-----	84.0		9	11.45 a. m.-----	107.0	
3	11.30 a. m.-----	94.0	Increased.	10	11.50 a. m.-----	108.0	
4	11.34 a. m.-----	100.0		11	12.00 m.-----	109.0	
5	11.36 a. m.-----	103.0		12	12.15 p. m.-----	108.5	
6	11.38 a. m.-----	104.0		13	1.00 p. m.-----	107.0	
7	11.41 a. m.-----	105.0					

### SUMMARY

A pile of heating stable manure was observed to ignite "spontaneously." When exposed to the air, charred straw glowed a fiery red. Oxygen aeration of a small section of the heating material produced a rapid increase in temperature of 26.5° C. in 30 minutes.





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## A CYTOLOGICAL STUDY OF PUCCINIA GLUMARUM ON BROMUS MARGINATUS AND TRITICUM VULGARE<sup>1</sup>

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### INTRODUCTION

Stripe rust, *Puccinia glumarum* (Schm.) Eriks. and Henn., was discovered in America in 1915, and a series of investigations of the rust have been carried on here since that date. Humphrey, Hungerford, and Johnson (23)<sup>3</sup> published in 1924 an account of its discovery in America, its geographic range and host range, its relations to climate, and its economic importance. Hungerford (24) recorded data on overwintering and oversummering, viability of spores, and experiments proving that the disease "is not transmitted from one wheat crop to the next by means of infected seed wheat." Hungerford and Owens (25) in 1923 carried on extensive inoculations of *P. glumarum* on grasses and grains, particularly wheat, to determine the host range and physiologic forms of the rust and also to determine degrees of susceptibility and resistance in the hosts. The present paper adds to these investigations of stripe rust in America a cytological study of the uredinial and telial stages.

In "Die Getreideroste" in 1896, Eriksson and Henning (20) recounted their experiments which led to the division of *Puccinia rubigo-vera* (DC.) Wint. into several species, one of which was *P. glumarum*. This account included a description of *P. glumarum* with details of uredinia and telia on leaves, stems, heads, and seed of wheat. They found several distinct physiologic forms of the rust on different grains and grasses.

The mode of overwintering of the rust was obscure. The teliospores could germinate either in the fall or in the following spring. No aecial host was discovered, and the authors doubted the existence of an alternate host. Attempts to infect wheat with sporidia failed, but the authors did not consider the possibility excluded. Stripe rust is very resistant to cold, but its winter survival in Sweden by either urediniospores or uredinio-mycelium on young wheat plants was too rare to explain the sudden and widespread outbreaks of rust in the spring. Moreover, they found the germination of uredinio-

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 511.

spores to be poor and capricious ("launenhaft"), and believed that the spread of the rust from any one infection center was very limited. This led them to suggest the possibility of dormant mycelium in the seed as a means of overwintering and an explanation of the outbreaks of rust in the spring.

In pursuance of this idea, Eriksson (14, 15) grew grain from rusted seed in small glass cases to prevent infection from without. Notwithstanding this, stripe rust appeared on the inclosed plants. No mycelium was found in very young seedlings, however, and Eriksson concluded that the "Krankheitsstoff" must be hidden and latent in the host cells in the form of an intimate symbiotic mixture of fungous and host protoplasm which he called "Mycoplasma." Only when outer conditions—soil and weather—were favorable did the fungous plasm separate itself from the host protoplasm, leave the host cells, and form ordinary intercellular mycelium.

Drawings representing the corpuscles of fungous plasm ready to leave the host cells were published in 1902 (16). These were followed by a series of illustrated cytological studies (17) giving details of the "dickem Plasma" in host cells near the borders of infections; of the swelling and dissolution of the host nucleus due to the fungus; the formation of "Plasmanucleoli" of fungous matter; the emptying of these nucleoli into intercellular spaces through fine tubes, giving rise to irregular masses of fungous plasm without septa and without nuclei; and finally of the formation of ordinary hyphae from these formless intercellular masses. The hyphae figured are coarse, multinucleate, and nonseptate.

Eriksson's mycoplasm hypothesis gained little support, but he reiterated his faith in it in 1905 (18) and again in 1921 (19).

Much of the later cytological work on stripe rust centered on the question of mycoplasm. In 1900 Klebahn (28) noted that the stripe-rust hyphae are few and coarse and multinucleate and can be traced along the length of the leaf for considerable distances. Near a uredinium the hyphae have more cross walls and fewer nuclei. He figured and described the haustoria and surmised that they were the "special corpuscles" or "Plasmanucleoli" forming the basis of Eriksson's mycoplasm hypothesis. In 1904 Klebahn (29) published figures of stripe rust showing host cells filled with "dickem Plasma" and containing numerous fungous nuclei and also a rust hypha containing a host nucleus. He regarded these, however, as artifacts ("Kunstproducte") and continued skeptical of the mycoplasm theory.

Ward, in England, was an able antagonist of the mycoplasm hypothesis. In 1903 (37) he published a cytological study of the uredinal generation, using principally *Puccinia dispersa*, and traced the development through from the germination of the spore. Ward contended, and correctly, that Eriksson's "corpuscles spéciaux" were haustoria and that Eriksson reversed the order of events in stating that they form in the host cell from the hypothetical mycoplasm and then move out into intercellular spaces to form hyphae.

In 1905 Ward (38) again discussed mycoplasm, reporting experiments to show the great longevity of urediniospores, their broad distribution by wind, the conditions governing their germination, and the probability of their overwintering. He described the germ tube, the multinucleate substomatal vesicle, and the large, multinucleate, nonseptate hyphae of *Puccinia glumarum*. He found no evidence of



the procedure described by Eriksson. The appearances of *P. glumarum* on a resistant wheat and on a susceptible wheat deprived of carbon dioxide are similar. In both, the hyphae soon become smaller and starved looking, their nuclei become indistinct, haustoria are rare, and host cells collapse and stain red.

Marryat (31) in 1907 studied cytologically the development of *Puccinia glumarum* on susceptible and immune varieties of wheat. On the susceptible host the germ tube enters without forming an appressorium. The vesicle has two nuclei and the stout infecting hypha four. Later hyphae are large and multinucleate, and haustoria are small and globular. About the tenth day "the nuclei now arrange themselves in pairs along the length of the hyphae which become divided up by septa." Spores are binucleate and are produced in abundance. On a resistant host entry is similar, but the contents of the hyphae soon become watery and then die, staining deep red. Haustoria are rare and minute, and host tissues die.

Pole Evans (21) in 1907, working in Ward's laboratory, found in *Puccinia glumarum* a cylindrical, multinucleate, substomatal vesicle which forms at one end a single infecting hypha. The appressorium was mentioned but not figured. The short branches of the mycelium are frequently septate, and the long branches are vacuolated, rarely septate, and apparently do not form haustoria. Long hyphae will grow 12 mm., then branch at the apex and start a new uredinium. Haustoria may be simple or branched and often contain as many as five nuclei. After a haustorium has formed from the tip of a hypha, a transverse septum makes its appearance and cuts off the tip. Later, many nuclei of the hyphae degenerate, and hyphae become very thin. Septation occurs the day before spore formation.

Lindfors (30) in 1924 studied cytologically plants bearing the first outbreak of rust in the spring and also later infections. The two were essentially alike; he found no evidence of mycoplasma. The binucleate spore forms a germ tube with two nuclei. The vesicle is thick walled and pushes out divergent infecting hyphae. Later, hyphae shape themselves to the space they occupy and are thin walled and multinucleate. Still later, long runners develop with septum formation at the branches. Then follows septation into binucleate cells, accompanied by degeneration of some of the nuclei. As reproduction begins, the nuclei of the mycelium become faint and the cytoplasm scant. The mycelium is to be considered as binucleate in principle because it returns to the binucleate condition during reproduction.

From the foregoing, it is evident that the cytological studies of stripe rust have been fragmentary and frequently at variance.

Eriksson's experiment of growing rusted plants from rusted seed in closed glass cages excited widespread interest. The experiments were repeated in Germany by Klebahn (27), in England by Massee (32), in Austria by Zukal (40), and in America by Bolley (11), and later by Hungerford (24), with uniformly negative results. The plants from rusted seed, grown in rust-proof cages, remained rust free.

Beauverie (6) in 1914 found that spores of *Puccinia glumarum* in seeds remained viable until February, and he considered it possible that the rust overwintered by this means.

During this time, however, evidence was accumulating throughout Europe that stripe rust can overwinter as hibernating mycelium in

leaves of grain and grasses. Spore production stops during cold weather, but, if the host survives, the mycelium produces fresh spores in the spring. This evidence was compiled by Henning in 1919 (22).

Attempts have been made to determine differences in chemical composition between varieties of wheat resistant and those susceptible to stripe rust. Henning and Bygden (22) in 1919 determined the acid and sugar content of several wheat varieties. No correlation was observed between either acid or sugar content and resistance. The most susceptible varieties had an exceptionally high sugar content, but so did Thule wheat, which was resistant. Arrhenius (5) in 1924 found no connection between the titration acidity and resistance to yellow rust.

Biffen (9, 10) made crosses between varieties of wheat immune from yellow rust and varieties susceptible to it. The  $F_1$  generation was susceptible. The  $F_2$  consisted of three susceptible to one immune. The immune plants bred true in succeeding generations. The others presented considerable range in susceptibility, but not more than would be accounted for by differences in manuring. In the  $F_3$  they showed again a 3-to-1 ratio. Rust resistance was considered due to a single factor, inherited independently of all the morphological characters studied. Brooks (12) in 1921 and Armstrong (4) in 1922 were in substantial agreement with this.

Nilsson-Ehle (33), on the contrary, found transgressive segregation when varieties of different rust resistance were crossed and even when varieties of equal rust resistance were crossed, and he believed that several independent factors were concerned in producing the results. Pesola (34) also found evidence of more than one factor, and suggested that the varying results of investigators in different countries might be due to their working with different forms of the rust. Substantial progress in breeding rust-resistant varieties has been made by Biffen in England, Nilsson-Ehle in Sweden, Pesola in Finland, and others.

#### MATERIAL AND METHODS

Spores of stripe rust and seed of Jenkin club wheat were obtained from C. W. Hungerford, of Moscow, Idaho. Plants were grown and infected, and material fixed.

Later, stripe rust was found growing on *Bromus marginatus* Nees (E. 25, T. O. 780) in the grass garden in Berkeley, Calif., in the summer of 1926. The stripe rust was dormant, although another rust on the same plants was growing actively. When the fall rains came the stripe rust renewed its growth and the fresh spores were transferred to seedlings of *B. marginatus*. Transfer to several varieties of wheat showed that it made normal growth. The rust probably was *Puccinia glumarum tritici*.

In inoculating, rain water was used for spraying the plants. Neither tap water nor distilled water produced good results. The plants were kept in a moist chamber for 48 hours after inoculation, then placed under cheesecloth cages in greenhouse and field. The highest greenhouse temperatures of summer (90°–105° F.) proved fatal to the rust, although the hosts survived.

*Bromus marginatus* has proved a favorable host for culturing the rust, as the individual leaves are long lived, and a single infection can live and continue growth for five, six, and even seven weeks. Chains of uredinia reach a length of 10 and 11 cm. If the plants are kept well watered, fresh spores are always available.

In the spring of 1926, stripe rust appeared in the wheat plots at Davis, Calif. Material was fixed on April 22. At this time several rows in the identification nursery were heavily infected, and scattered infections had appeared at a number of other points in the field. Material was fixed from row 162 (C. I. 7554, a wheat from Scotland), row 163 (C. I. 7555-1, a wheat from India), row 165 (like 163), row 166 (C. I. 7556, a wheat from Algeria), and White Federation (C. I. 4981). Later on, May 14, 1926, telia on these same hosts were fixed. It was interesting to observe that although the rust had been growing and spreading vigorously when seen in April, it had made no further headway by the middle of May. The hotter, drier weather of May had checked its spread. Other wheat rusts, on the contrary, were increasing rapidly at the later date. Beauverie (?) made similar observations on stripe rust in France. The temperature range of stripe rust appears to be distinctly lower than that of the other wheat rusts.

Material was fixed in the chrom-acetic-urea mixtures and in Flemming's medium and weak solutions. It was washed and dehydrated by the usual methods, embedded in 50° paraffin, and the sections were cut 10 $\mu$  thick and were usually stained in the triple stain. Hanging drop cultures of germinating spores were fixed over fumes of osmic acid, stained, and mounted in balsam.

#### INVESTIGATIONS

The urediniospores of *Puccinia glumarum* are bright yellow in color. Under the microscope the color is found to be in the cell contents. The wall is colorless. When mounted dry the spore wall is seen to be minutely echinulate. The average size is 22.5 $\mu$  by 17.4 $\mu$ . In water, the spores swell in a few seconds, becoming more nearly globular as they enlarge, averaging 29.5 $\mu$  by 27.1 $\mu$ . The stretched walls are thinner, of course, and are under considerable tension. Under slight mechanical pressure the spores burst and the empty walls contract, but not quite to the original size, at least not while remaining in water. They now average 24.6 $\mu$  by 19.8 $\mu$ .

In the spore wall are scattered germ pores. They range from 10 to 16 in a spore, and the average of 20 is 13.5. These are obscure in the living material but are readily brought out by staining. They are clearest in germinated spores whose contents have passed out into the germ tube. (Pl. 1, A, B, C.) In a preparation stained with gentian violet (pl. 1, B) the germ pores appear as small lighter-stained circles. When stained with safranin (pl. 1, C, *a*, *b*) each pore has a darker staining center. In edge view (pl. 1, D, *a*, *b*, *c*) the germ pores appear as thickened pads of light-staining material.

The yellow color of the spore contents is lost in fixed and sectioned material, and if the spore wall has not been too heavily stained the contents of the spore can be made out. (Pl. 1, D.) These consist of rich cytoplasm and usually two nuclei.

#### SPORE GERMINATION

Under favorable conditions the spores germinate in a few hours. On the leaf, where moisture conditions are not uniform, some spores may germinate at once, others not until several days after inoculation. The germ tubes follow closely the irregularities of the leaf surface, and in sectioned material it is seldom possible to follow a single germ tube for any distance. The growing tip of such a tube is seen in

Plate 1, E. It is uneven in thickness and somewhat swollen near the tip, *b*, where two nuclei lie close together.

In a hanging drop culture 1 day old, fixed and stained, the process of germination can be traced. An early stage is drawn in Plate 1, A. From one of the germ pores, *a*, the tube, *b*, issued. It is filled with dense, alveolar cytoplasm throughout its length. No nuclei are visible; they probably are still within the spore wall. A slightly longer germ tube is represented in Plate 1, B. The spore and the proximal part of the germ tube are nearly empty. At the growing tip, *b*, the cytoplasm is dense, and back of it at *a* is a pair of nuclei.

The great majority of the germ tubes in one-day hanging drop cultures are much longer than this. The average of 10 which were measured is  $980\mu$ . The maximum is  $1,428\mu$ . In these longer tubes the basal portion (pl. 1, C) is empty, and practically all of the protoplasm is concentrated at the tip (pl. 1, F, G). Usually only one pair of nuclei is to be seen (pl. 1, F, *a*), but more rarely two pairs occur (pl. 1, G, *a*, *b*).

On the leaf, the germ tubes grow directly to the stomata. No well-marked appressorium, such as occurs regularly in the stem and leaf rusts of wheat, has been seen in stripe rust. On reaching the stoma, the germ tube swells slightly. (Pl. 2, A, *a*.) This occurs in material fixed one day after inoculation. The cytoplasm is dense at the tip and thins out gradually farther back, as would be the case in the germ tube before it reached the stoma. No septum has formed to isolate a terminal cell.

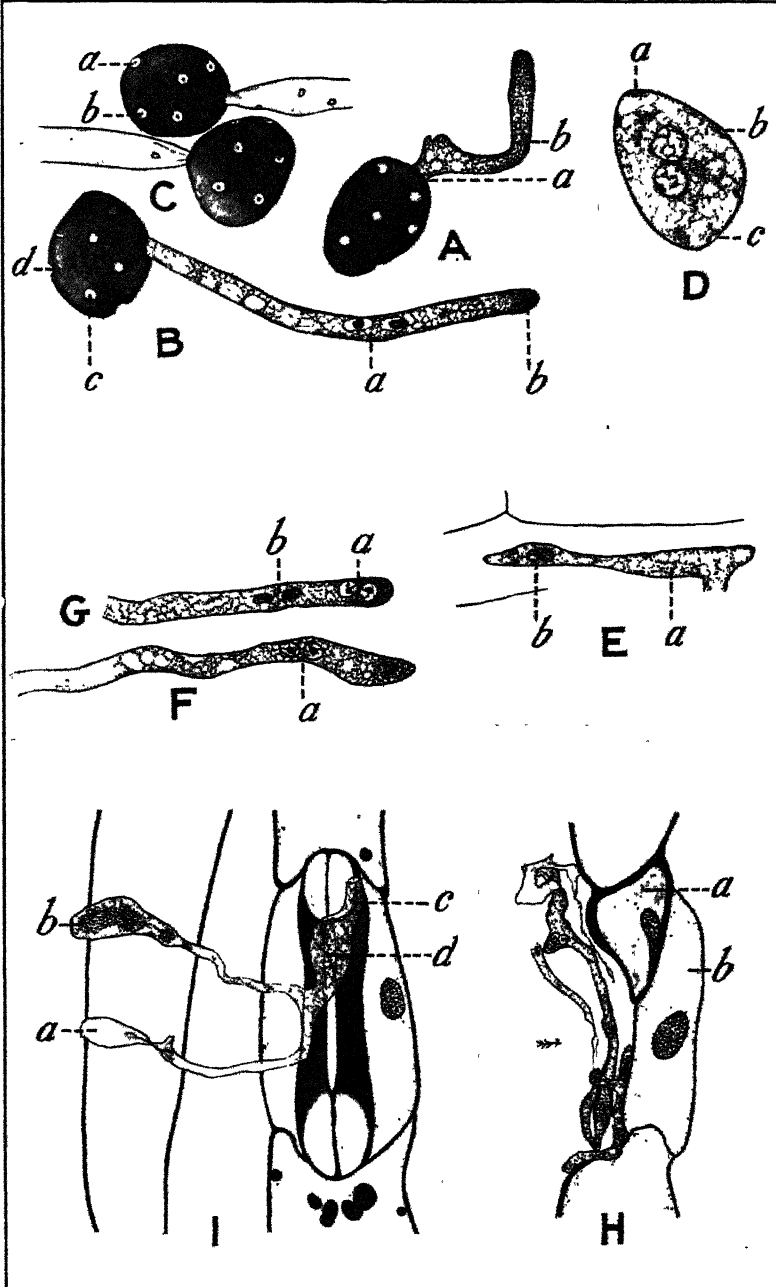
#### ENTRANCE OF THE FUNGUS

Entry of the fungus is usually very prompt, but even when it is delayed the fungous plasm does not round up into a cushion over the stoma. In Plate 1, H, from material fixed three days after inoculation, is represented a somewhat oblique section through a stoma, cutting through the end of a guard cell at *a*, and an accessory cell of the stoma at *b*. Several germ tubes have reached the stoma and, having failed to enter, have twisted about on its surface. No true appressoria have formed.

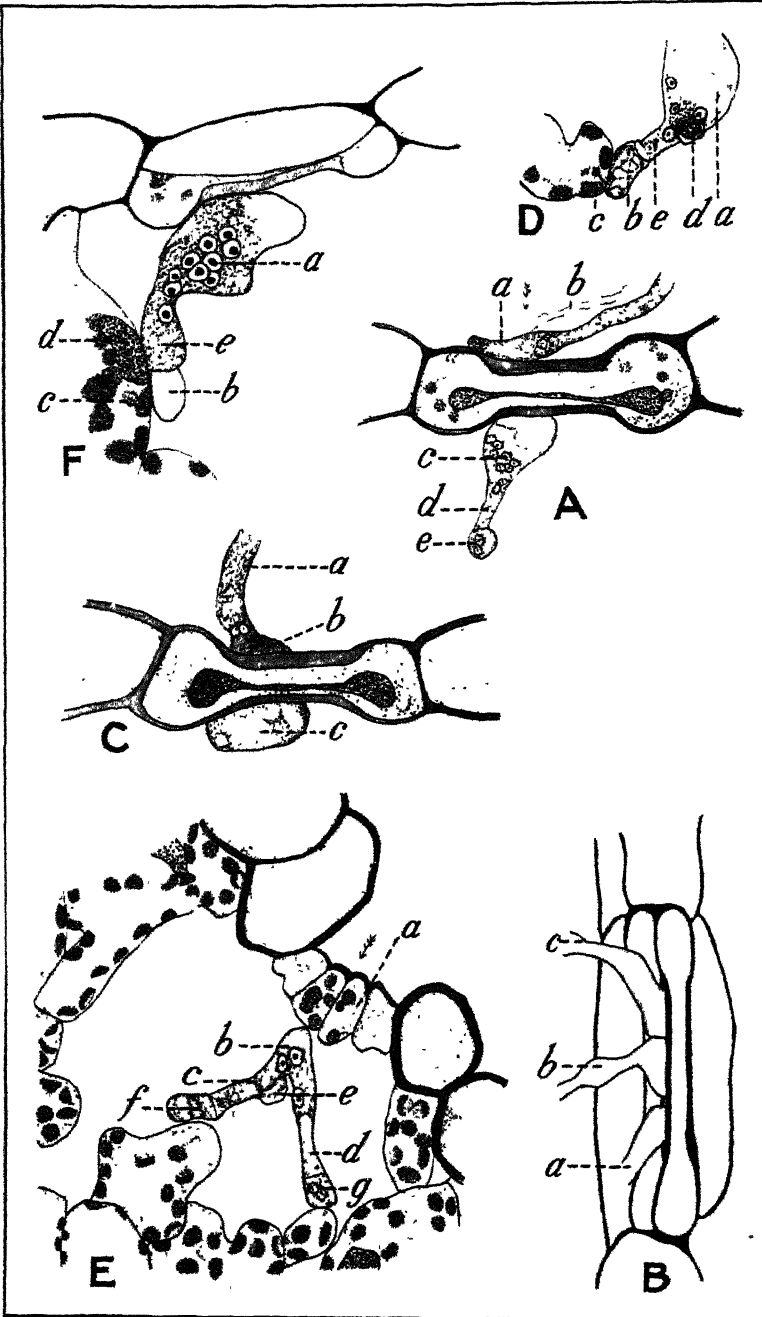
In a previous study of leaf rust (*Puccinia triticina*) (3), it was found that in a small percentage of cases where several germ tubes reached the same stoma the appressoria formed there might fuse, or might enter separately and fuse after entry. No conclusive evidence of a similar process has been seen in stripe rust, although some of the material suggests it. In Plate 1, I, is shown a surface view of a stoma on which three germ tubes, *a*, *b*, and *c*, converged at *d* where entry is in progress. The central fungous mass at *d* is apparently continuous, but the dark-stained guard cell walls underneath make it difficult to be sure of this. If fusion occurs it is rare, in this material

#### EXPLANATORY LEGEND FOR PLATE 1

- A.—Hanging drop culture. The spore, *a*, has formed a short germ tube, *b*. Germ pores distinct.  $\times 730$ .  
 B.—Later stage of germination. The germ pores, *c*, *d*, show clearly on the empty spore. The cytoplasm of the germ tube is densest at the tip, *b*. Two nuclei at *a*.  $\times 730$ .  
 C.—Still later. Spore and basal part of tube empty. Germ pores at *a* and *b*.  $\times 730$ .  
 D.—Spore showing contents—two nuclei and cytoplasm. Germ pores, *a*, *b*, *c*, distinct.  $\times 1130$ .  
 E.—Tip of germ tube on leaf showing pair of nuclei, *b*, and vacuolate cytoplasm farther back at *a*.  $\times 730$ .  
 F.—Tip of old germ tube from hanging drop culture. Two nuclei at *a*. Basal part nearly empty.  $\times 730$ .  
 G.—Similar. Two pairs of nuclei, *a* and *b*.  $\times 730$ .  
 H.—Three days after inoculation of *Bromus marginatus*. Germ tubes reached stoma but failed to enter. Portion of guard cell at *a*, and accessory cell at *b*. No appressoria formed.  $\times 730$ .  
 I.—Four days after inoculation on *B. marginatus*. Three germ tubes, *a*, *b*, *c*, converged at the stoma at *d* and are entering, apparently as one mass.  $\times 730$ .



(For explanatory legend, see page 492)



(For explanatory legend, see page 493)

at least. Ordinarily when several germ tubes reach the same stoma they remain separate. In Plate 2, B (drawn in outline only), the three germ tubes, *a*, *b*, *c*, are distinct.

Entry takes place immediately if conditions permit. There is ordinarily no pause at the stoma. In Plate 2, C, drawn from material fixed only 16 hours after inoculation, entry has begun. It usually takes place, as in this case, at one end of the stomatal slit. The germ tube reached the stoma at *b*, spread slightly along the crack between the guard cells (only one of which is drawn), pushed through, and is swelling out into a rounded vesicle at *c*, on the inner surface of the stoma. The bulk of the protoplasm is still outside in the germ tube, *a*, *b*, where the contents are relatively dense.

In the example just described, the germ tube spreads somewhat along the stoma before entering, but this is not essential. The unmodified tip of the germ tube can enter directly, merely becoming somewhat flattened as it passes through the narrow slit.

Here, too, no septum can be seen in the germ tube. (Pl. 2, C.) It may be just this lack of a delimiting wall which prevents the formation of the large rounded appressorial cushion typical of other rusts. A little later, however, when entry is completed, a septum occasionally may be seen. (Pl. 4, A, *f*.) It is not uniformly present.

#### FORMATION OF INITIAL HYPHA AND HAUSTORIUM

Plate 2, A, is drawn from material fixed 24 hours after inoculation. Two germ tubes reached this stoma. The one, *a*, already mentioned, came last. Another arrived earlier and has entered. An empty withered germ tube, *b*, is all that remains of it outside the stoma. Inside are the substomatal vesicle, *c*, and a short hypha, *d*, swollen at the tip, *e*, in preparation for the formation of a haustorium. The cytoplasm is rather thin and the turgor low. Nuclear divisions have evidently occurred, for there are now 10 or 12 small nuclei.

In the case just described, a single hypha formed from the substomatal vesicle. More commonly there are two or three. As they extend in divergent directions, only a fraction of the whole occurs in any one section. In Plate 2, D, drawn also from one-day material, the partly evacuated vesicle, *a*, gave rise to two hyphae, one at *e*, which has formed a haustorium mother cell, *b*, and the beginning of a haustorium at *c*. The base of a second hypha is at *d*, the rest having been cut off in sectioning.

Another view of this stage is drawn in Plate 2, E, which is a detail from a cross section of a leaf fixed 24 hours after inoculation. Underneath the cross section of the stoma, *a*, is the large substomatal air chamber bounded by mesophyll tissue. In this air space is the end

#### EXPLANATORY LEGEND FOR PLATE 2

##### Infections on *Bromus marginatus*

A.—One-day infection. One germ tube, *a*, has just reached the stoma. The second, *b*, is empty, the contents having entered to form the substomatal vesicle, *c*, which in turn has formed a short infection hypha, *d*, terminated by a haustorium mother cell, *e*.  $\times 730$ .

B.—Sixteen hours after inoculation. Three germ tubes, *a*, *b*, *c*, (outline only) reached the same stoma. No appressoria formed, and there is no fusion.  $\times 730$ .

C.—Early stage of entry. The germ tube, *a*, reached the stoma at *b*, and is passing through to form the vesicle, *c*.  $\times 730$ .

D.—One-day infection. The substomatal vesicle, *a*, pushed out two hyphae; one at *d*, sectioned, and a second, *e*, which has formed the haustorium mother cell, *b*, and the beginning of a haustorium *c*.  $\times 730$ .

E.—One-day infection. Cross section showing stoma, *a*, end of vesicle, *b*, two infecting hyphae, *c* and *d*, terminating in haustorium mother cells, *f* and *g*. A third infecting hypha, *e*, was cut in sectioning.  $\times 730$ .

F.—Two-day infection. Enlarging substomatal vesicle at *a*. Empty haustorium mother cell, *b*, and end of haustorium, *c*, accompanied by host nucleus, *d*. Hypha swelling at *e*.  $\times 730$ .

of the substomatal vesicle, *b* (the body of which is in an adjoining section), and two hyphae, *c* and *d*, each terminating in a haustorium mother cell at *f* and *g*, respectively. At *e* is the stub of a third infecting hypha, cut off in sectioning. Each of the haustorium mother cells is delimited by a septum and contains cytoplasm and two small nuclei. No other septa are to be seen. The number of nuclei in the first-formed haustorium mother cell is not uniform. Three and even four have been found, although two are most common.

The substomatal vesicles are small at this stage and meager in content. The average size of 10 is  $17.5\mu$  by  $12.3\mu$ . The longer diameter is often at right angles to the epidermis, as in Plate 2, A and D.

Under winter conditions (this material was infected in January) the rust spreads little if at all on the second, third, and fourth days after inoculation. It still is limited to the edges of the air space beneath the stoma of entry. Development during this time consists in the absorption of food from the host, an increase in cytoplasm, and multiplication of nuclei accompanied by a swelling of the vesicle and hyphae to several times their original dimensions.

The steps in this process can be followed. Two-day material presents considerable range in development. One of the early stages is shown in Plate 2, F. It is drawn on the same scale as the younger vesicles and hyphae in Plate 2, A and D. On comparing the two, the trend of development is at once apparent. The stage in Plate 2, A, has been attained without food other than that originally stored in the spore from which it grew. In Plate 2, F, there is an empty haustorium mother cell at *b* and the tip of a haustorium at *c*. The remainder of the haustorium lies in the next section. Once the food and water supplies of the host have been tapped, there is immediate expansion. Both vesicle and hypha are rounding out. The nuclei have not increased in number but are full grown.

Food absorbed from the host cell by the haustorium and passed on out through the mother cell is naturally most abundant just back of this cell. Here fungous plasm accumulates. There is formed regularly a rounded, almost globular swelling of the hypha at this point. The beginning of this swelling is seen at *e* in Plate 2, F. It is more pronounced at *a* in Plate 3, A, just back of the mother cell *b*. In Plate 3, *B*<sub>1</sub> and *B*<sub>2</sub>, are drawn successive sections of the same fungus, which has formed two haustoria. Just outside of the host cell containing the haustorium, *g*, is the beaklike haustorium mother cell, *f*, and just back of it is the rounded swelling of the hypha *e*. In Plate 3, *B*<sub>2</sub>, is the haustorium, *d*, connected with the mother cell, *c*, and back of it a large globular fungous mass, *b*. This connects with the part labeled *b* in *B*<sub>1</sub>. The point at *a* connects directly with

#### EXPLANATORY LEGEND FOR PLATE 3

##### Infections on *Bromus marginatus*

A.—Two-day infection with partly collapsed substomatal vesicle, *c*, and infecting hypha, *a*, greatly swollen just behind the haustorium mother cell, *b*. Haustorium at *d*.  $\times 730$ .

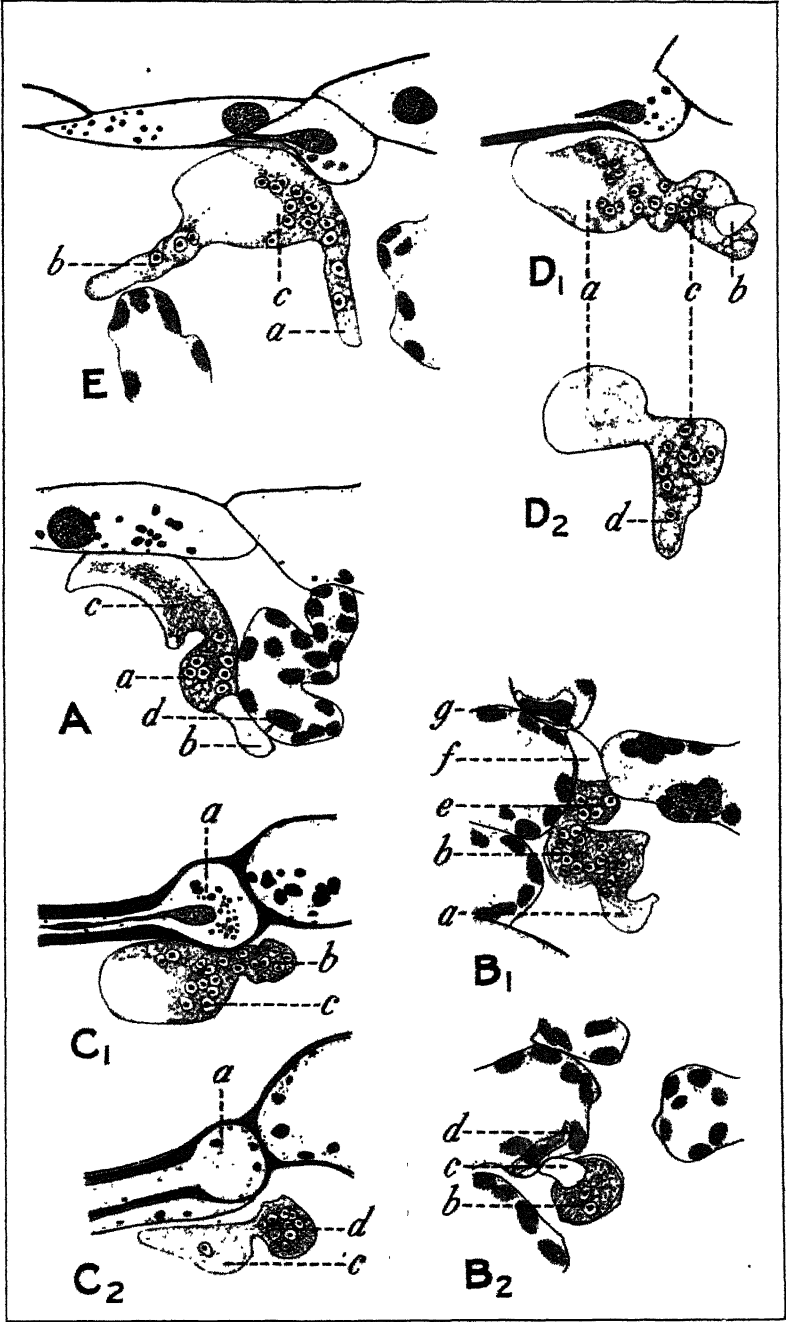
*B*<sub>1</sub> and *B*<sub>2</sub>.—Successive sections of a two-day infection. *B*<sub>1</sub>, *a*, adjoins the substomatal vesicle (not drawn). There are two infecting hyphae, greatly enlarged. One at *B*<sub>2</sub>, *b*, with empty haustorium mother cell at *c* and haustorium at *d*. The second, *B*<sub>1</sub>, *e*, with mother cell at *f* and haustorium, *g*.  $\times 730$ .

*C*<sub>1</sub> and *C*<sub>2</sub>.—Two-day infection. Successive sections of substomatal vesicle showing increase in size and in contents. Guard cell at *a*, substomatal vesicle, *c*, swollen infecting hypha, *b*, *d*.  $\times 730$ .

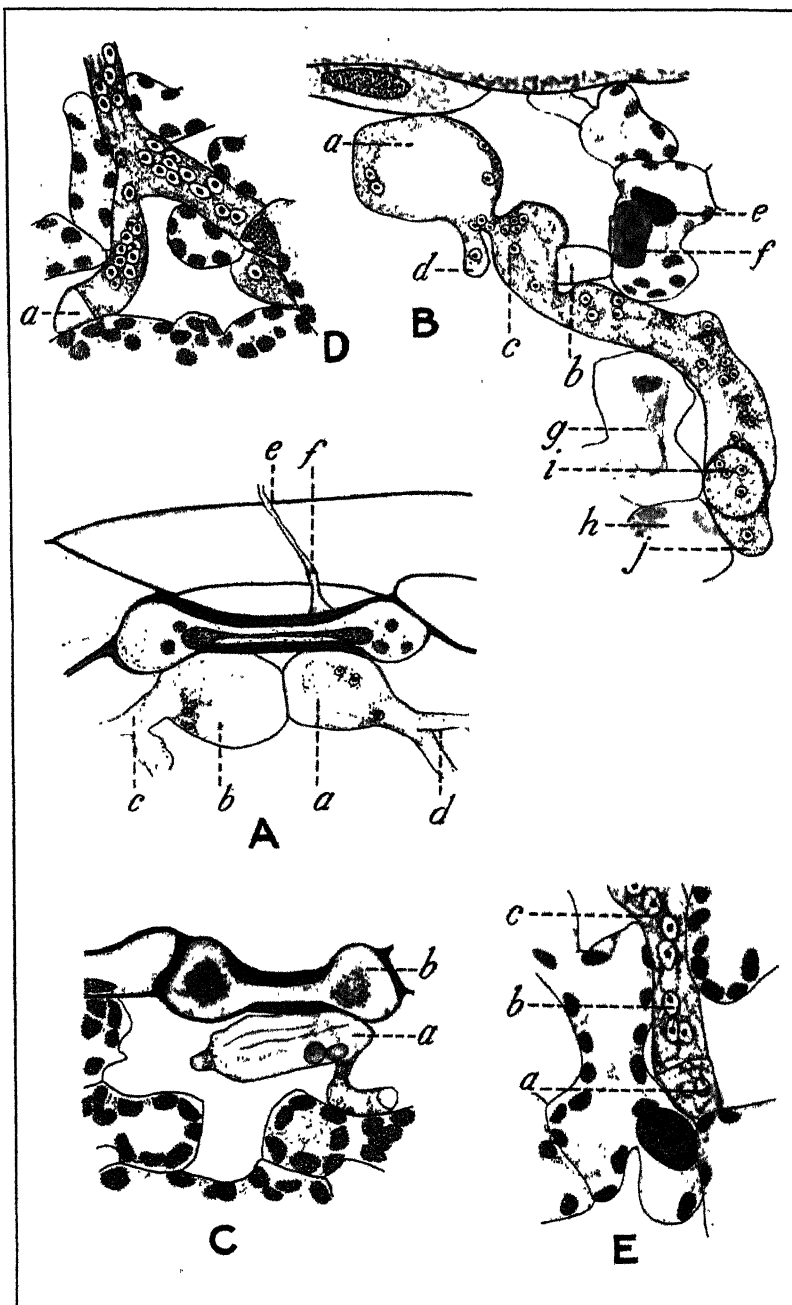
*D*<sub>1</sub> and *D*<sub>2</sub>.—Four-day infection showing the substomatal vesicle, *a*, the empty haustorium mother cell, *b*, the hypha greatly swollen just back of it at *c*, and the growing tip, *d*. No septa except at *b*.  $\times 730$ .

*E*.—Four-day infection with the substomatal vesicle, *c*, forming an infecting hypha at each end, *a* and *b*.  $\times 730$ .





(For explanatory legend, see page 494)



(For explanatory legend, see page 495)

the substomatal vesicle. There are over 2 dozen nuclei in this irregularly bulging mass. There are only two septa—the ones delimiting the two haustorium mother cells.

One result of the absence of cross walls is a greater freedom of motion. There are no nuclei in the short hypha at *d* in Plate 2, A, nor at *e* in Plate 2, F. A little later all of the nuclei are at the corresponding position, *a*, in Plate 3, A.

When the substomatal vesicle first rounds out in this process of expansion it is rather unstable and sometimes collapses under the action of the fixing fluid. (Pl. 3, A, *c*.) A little later it holds its form. Moreover, as the protoplasm grows and the swelling hypha is densely filled, the protoplasm often backs up into the vesicle, for there are no cross walls to stop it. In Plate 3, C<sub>1</sub> and C<sub>2</sub>, still from two-day material, the vesicle, *c*, is more than half full of cytoplasm rich in nuclei. It is several times its original size (cf. pl. 2, A and D) and has changed shape, for the long diameter is now regularly parallel to the long axis of the guard cells. Ten substomatal vesicles from three-day material have been measured. The average size is 26.8 $\mu$  by 17.1 $\mu$ , which contrasts sharply with the original size (17.5 $\mu$  by 12.3 $\mu$ ).

Similar figures still may be found on the fourth day. In Plate 3, D<sub>1</sub> and D<sub>2</sub>, successive sections of the same organism, there is the half-filled vesicle, *a*, against the guard cell. The haustorium mother cell, *b*, originally the tip of a slender hypha, looks now like a little lateral appendage on a short massive hypha whose apex is at *d*.

Although two or three hyphae may form from the same vesicle, they usually issue near each other from the same end of the vesicle. (Pl. 2, D and E.) Occasionally, however, one finds hyphae coming from opposite ends of the vesicle, as in Plate 3, E, *a* and *b*. So far as noted, the development of the two in such cases is unequal. As in this instance, the bulk of the cytoplasm in the vesicle is at one end, continuous with the hypha, *a*, which in turn connects (in adjoining sections not drawn) with the usual haustorium mother cells (two in this case) and globular swellings. There are over 40 nuclei in this fungus, of which only 3 occur in the hypha at *b*. The further development of the latter is doubtful.

Occasionally two germ tubes reach the same stoma and both enter. Figures like the one drawn in Plate 4, A, are not uncommon. At *e* is the empty germ tube leading to the vesicle, *a*. The survival of the germ tube to this stage (fourth day) is rare. It usually disappears soon after entry; in fact, it is rarely seen even on the second day. The two vesicles, *a* and *b*, are quite distinct, and each forms its own infecting hyphae, *c* and *d*. In this case the vesicles are nearly emptied, the cytoplasm and nuclei having passed out along the lines of growth.

#### EXPLANATORY LEGEND FOR PLATE 4

A.—A four-day infection on *Bromus marginatus*. Two entries occurred at the same stoma. The two nearly empty substomatal vesicles, *a* and *b*, are not joined. Each produced two infecting hyphae, *c* and *d*. Remnant of germ tube at *e*, *f*.  $\times 730$ .

B.—From a seven-day infection on *B. marginatus*. The substomatal vesicle, *a*, formed two hyphae; one abortive, *d*, the other forming a mother cell at *b* with dead haustorium, *f*, accompanied by dead host nucleus, *c*. The hypha expanded at *c*, and formed the nonseptate hypha, *c*, *j*, with branch at *i*. Dead host cells at *g*, *h*. Multinucleate, nonseptate mycelium from this extends 450  $\mu$ .  $\times 730$ .

C.—Seven-day infection on *B. marginatus* with empty withered substomatal vesicle at *a* beneath dead guard cell, *b*.  $\times 730$ .

D.—Seven-day infection on *B. marginatus*. Branching hypha in mesophyll. Nuclei irregularly arranged. No septa except those delimiting the haustorium mother cells, as at *a*.  $\times 730$ .

E.—From 17-day infection on Jenkin club wheat. Hypha, *b*, *c*, terminates in a haustorium mother cell, *a*.  $\times 1130$ .

The time when this evacuation of the vesicle occurs varies greatly. In Plate 4, A, four days after inoculation, it is nearly complete. In Plate 4, B, on the contrary, seven days after inoculation, cytoplasm and nuclei are still present, and the vesicle has retained its turgor. The evacuation of the vesicle is accompanied by its shrinkage. This is beginning in the vesicles of Plate 4, A, and is much more pronounced in later preparations. In Plate 4, C, seventh day, the vesicle is small and wrinkled, and contains mere traces of cytoplasm and nuclei.

The material used in this study of early stages of infection was grown in the field in January, and the question arose as to whether winter conditions, particularly low temperature, had retarded the growth. For comparison, a second lot of inoculations was made in April. The early stages resembled closely those of the winter material, both as to method and rate of development. All evidence available at present indicates that this prolonged juvenile period, during which the organism increases in thickness without adding materially to its length, is normal to this species.

#### DEVELOPMENT OF THE MYCELIUM

After this long preliminary period, during which the primary hypha or hyphae expand slowly to the diameter of later stripe-rust hyphae, a hypha grows out into the mesophyll tissue, working its way through the irregular intercellular spaces. The direction of growth is at first indifferent. It may grow lengthwise of the leaf, but it is quite as apt to grow transversely or diagonally.

In Plate 4, B, representing part of a seven-day infection, the substomatal vesicle at *a*, already mentioned, is living. Two hyphae formed from it, one at *d*, abortive, and a second which originally terminated in a haustorium mother cell at *b*. The haustorium from it, *f*, is now dead, and alongside it is the host nucleus, *e*, also dead. The haustorium evidently functioned, however, for the typical rounded swelling, *c*, formed just back of the mother cell, and from it grew the hypha, *c-j*. A branch at *i* (cut in sectioning) leads out into deeper tissues, where it has branched and spread lengthwise of the leaf through a distance of 450 $\mu$ . The entire mycelium is without septa other than those delimiting haustorium mother cells. This absence of barriers facilitates the transfer of materials along the hyphae and may explain why this hypha, *c-j*, can still contain rich protoplasm, although the host cells around it, *g*, *h*, *e*, are dead or dying.

Something of the habits of the mycelium may be seen from Plate 5, A, drawn from a six-day preparation. Coming in at *b*, the hypha passes under a mesophyll cell, reappears at *d*, grows down to *k*, nar-

#### EXPLANATORY LEGEND FOR PLATE 5

A.—Six-day infection on *Bromus marginatus*. Branching hypha, *b*, *k*, *l*, *m*, in mesophyll. Haustorium mother cells at *a*, *c*, *d*, *f*, and *i*. Haustoria at *e*, *g*, and *j*. Host-cell nucleus at *h*. No septa except for haustorium formation.  $\times 730$ .

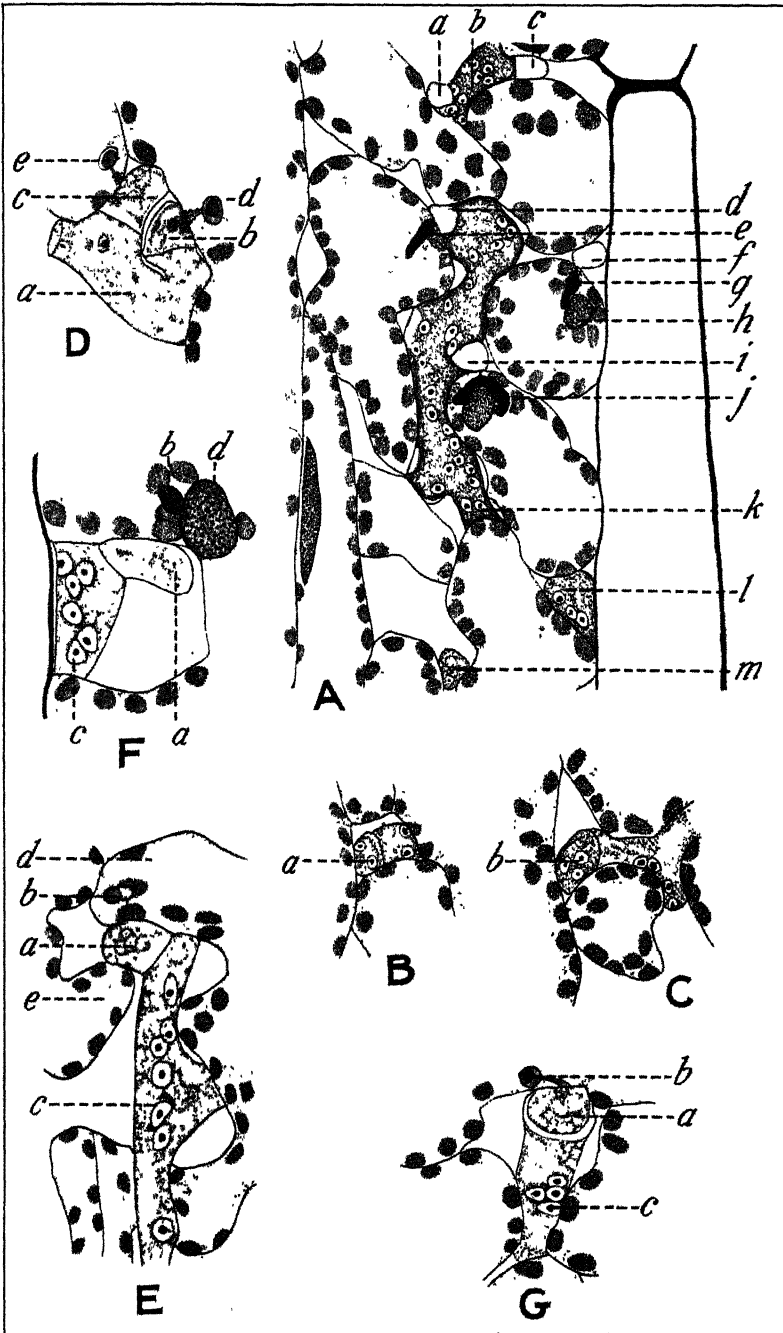
B and C.—From six-day infection of *B. marginatus*. Newly formed haustorium mother cells at *a*, *b*, one with two nuclei, the other with three.  $\times 730$ .

D.—From 17-day infection on Jenkin club wheat. Two haustorium mother cells, *b* and *c*, formed on the hypha, *a*, are beginning to form haustoria at *d* and *e*. The necks of these haustoria are encrusted with granular matter. The nuclei in the mother cells are indistinct.  $\times 1130$ .

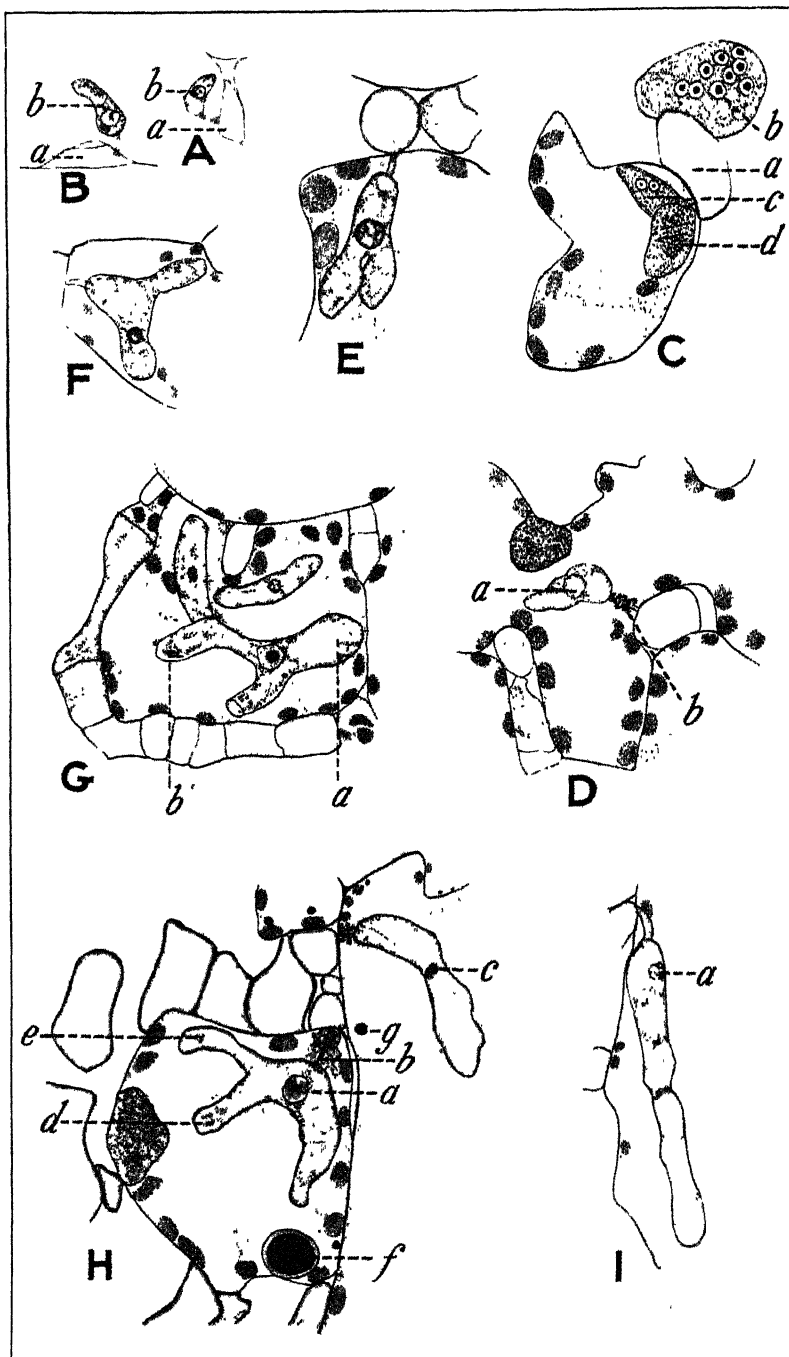
E.—Seventeen-day infection of Jenkin club wheat. The hypha, *c*, formed a mother cell, *a*, between the host cells, *d* and *e*. The mother cell is forming the haustorium, *b*. Nuclei of the mother cell are doubtful.  $\times 1130$ .

F.—Similar to E. On the hypha, *c*, has formed a haustorium mother cell, *a*. No nuclei visible in the mother cell. Young haustorium at *b*, with host nucleus, *d*.  $\times 1130$ .

G.—Similar to E. Hypha at *c*. Contents of mother cell, *a*, without visible nucleus. Young haustorium at *b*.  $\times 1130$ .



(For explanatory legend, see page 496)



(For explanatory legend, see page 497)

rowing where the air space narrows, broadening out where there is room, disappearing at *k*, and coming again into the plane of the section at *l* and *m*. Numerous nuclei are scattered along its length in no regular order. So far as can be detected, the nuclei are not paired.

The characteristic haustorium mother cells occur at Plate 5, A, *a*, *c*, *d*, *f*, and *i*, each connected with its haustorium as at *e*, *j*, and *g*. In *j* and *g* the host nucleus lies in contact with the haustorium. The tendency to form an expanded mass of fungous plasm in the hypha just back of the haustorium mother cell, where food is coming in, is less pronounced but can still be seen in some cases. In the hypha just back of the mother cell, *d*, is a large rounded mass almost filling the large air space. Food absorbed by the haustorium, *j*, and passed out through the mother cell, *i*, has brought about a local expansion of the hypha at that point, but space limitations have forced the swelling hypha around the mother cell itself, almost submerging it.

In larger air spaces the hyphae take a cylindrical form (pl. 4, D) and are far more uniform in thickness. The average diameter is about  $9\mu$ . Here, too, the fungous nuclei are distributed in irregular order, often with 10 or 12 in a group. There is no obvious pairing.

Hauatoria form in the same fashion in mycelia of all ages, but older infections have proved most favorable for a detailed study of all stages of the process. For some reason not understood, hauatoria of young infections often collapse in the fixing fluid even when the rest of the fungus and the host tissues are excellently fixed. In older infections some difference, probably in degree of turgor, resulted in much better fixation of hauatoria.

When the tip of one of these coarse multinucleate hyphae forms contact with a host cell or becomes wedged into an angle of some small intercellular space, a septum forms a short distance back from the tip, giving rise to a short broad terminal cell, the haustorium mother cell. (Pl. 5, B, *a*, and C, *b*.) The nuclei of this cell decrease rapidly and unequally in size and become very difficult to see. Only when the cell is first delimited can the nuclei be counted. In the first formed haustorium mother cells (pl. 2, E, *f* and *g*) the number of nuclei commonly is two. In Plate 5, B, *a*, there are two; in C, *b*, there are three. In other cases four, five, and even six have been counted. At a slightly later stage (pl. 4, E), although the numerous nuclei of the hypha, *b*, *c*, are clearly defined and conspicuous, those in the mother cell are small and vague. One little nucleus at *a* is fairly clear, but the smaller, fainter circles near it

#### EXPLANATORY LEGEND FOR PLATE 6

A.—From 17-day infection on Jenkin club wheat. Young haustorium beginning to expand with single nucleus at *b*. Empty mother cell at *a*.  $\times 1130$ .

B.—Similar to A. Empty haustorium mother cell, *a*, and uninucleate haustorium, *b*.  $\times 1130$ .

C.—From four-day infection on *Bromus marginatus*. Mother cell, *a*, with swollen hypha, *b*, behind it. The small haustorium, *c*, is binucleate and accompanied by the host nucleus, *d*.  $\times 1130$ .

D.—From 17-day infection on Jenkin club wheat. Expanding haustorium, *a*, with encrusted neck, *b*.  $\times 1130$ .

E.—From infection on leaf sheath of wheat, C. I. 7555-1. Half-grown, branched, uninucleate haustorium.  $\times 1130$ .

F.—From infection on leaf blade of wheat C. I. 7554. Forked haustorium with divergent branches  $\times 1130$ .

G.—From infection on glume of wheat C. I. 7555-1. Several haustoria in one cell. Haustorium, *a*, *b*, slightly plasmolyzed at *b*. Host cell living.  $\times 1130$ .

H.—From infection on leaf sheath of wheat C. I. 7555-1. Haustorium, *a*, with three branches slightly plasmolyzed at *d* and *e*, and heavily sheathed neck, *b*. Second haustorium at *c*. Intracellular bodies at *f* and *g*.  $\times 1130$ .

I.—From infection on leaf sheath of wheat C. I. 7555-1. Large haustorium ( $39\mu$  long) almost completely drained. Single haustorial nucleus at *a*.  $\times 1130$ .

may be either degenerating nuclei or merely heavier bits of cytoplasmic reticulum.

In Plate 5, E, the end of the hypha became firmly wedged between two host cells, *d* and *e*, and formed the mother cell, *a*. Within it are four small circles. Whether any or all of these are nuclei can not be determined. In this case, part of the content of this cell has moved in to the host cell, beginning the formation of a haustorium at *b*.

In Plate 5, F and G, the nuclei in the mother cells, *a*, *a*, either have become so faint or so small as to be indistinguishable or have already entered the haustoria, *b*, *b*.

In Plate 5, D, the hypha *a* has produced two mother cells, *b* and *c*, crowded into a small intercellular space and shaped closely to its outline. The one at *b* is forming the haustorium *d* and the one at *c* is entering another host cell at *e*. The necks of these young haustoria, *d* and *e*, are not slender like the others depicted, but are seemingly encrusted by granular matter. Whether this coating is derived from the host or from the rust is not known, nor is it known what conditions lead to its formation. It occurs more commonly in old than in young infections.

The very young haustorium is too dense and stains too deeply to show the nature of its contents. Apparently the protoplasm of the mother cell becomes concentrated as it flows through the narrow neck of the haustorium. It soon absorbs water from the host, however, and expands into a more open vacuolated structure. In Plate 6, A, at *b*, is a young haustorium only  $4.5\mu$  long, which shows clearly a single well-formed nucleus and reticulated cytoplasm. Its mother cell, *a*, is empty. The slightly older haustorium (pl. 6, B, *b*),  $7\mu$  long, also shows a single clearly defined nucleus. Whatever the number of nuclei in the mother cell may have been, but one reappears in the haustorium, and it expands with the growth of the haustorium. A few exceptions have been noted. The haustorium at *c*, in Plate 6, C, contains two small nuclei. The fate of the extra nuclei when a uninucleate haustorium arises from a binucleate or multinucleate mother cell has not been determined, but the fact that the nuclei decrease unequally in size before entering the haustorium suggests degeneration of the supernumerary nuclei.

The great majority of the haustoria develop into elongated unbranched bodies, thicker at the base and tapering somewhat toward the tip. The single nucleus lies about one-third of the way from the base. In Plate 6, D, the half-grown haustorium, *a*, is typical. Here again the neck, *b*, is encrusted with a blue-staining granular material of unknown origin. This occurs not infrequently in infections on a number of hosts, but there always are many haustoria with slender necks along with those that show this modification. It is doubtful whether it should be considered a sign of resistance in the host. Plate 6, D, was drawn from an infection on Jenkin club wheat, on which the rust makes good growth. It is found equally commonly on *Bromus marginatus*, a fully susceptible host; also on C. I. No. 7555-1, a susceptible wheat from India.

Occasionally a haustorium forks into two branches which may lie close together (pl. 6, E) or diverge (pl. 6, F and G, *a*). More rarely three branches are formed. (Pl. 6, H, *a*.) The single nucleus of these branching haustoria is usually stationed near the point of



origin of the branches. This last haustorium (pl. 6, H, *a*) has an unusually thickened neck, *b*, within which the slender original neck can be seen. Occasionally there is a slightly denser mass of cytoplasm near the tip of a branch of the haustorium (H, *d*, *e*). In case the haustorium is slightly plasmolyzed (pl. 6, G, *b*) this mass may bear a superficial resemblance to the haustorial nucleus. Usually, however, it is irregular enough in outline to be unmistakable.

Full-grown haustoria are  $25\mu$  or  $30\mu$  in length (counting each branch separately). The largest one measured (pl. 6, I) is  $39\mu$  long.

The host nucleus is often found associated with the haustorium. A count was made to determine the percentage of cases in which the haustorium and nucleus were together. Only those were counted in which the haustorium was full grown and in which both nucleus and haustorium could be seen clearly, and in mesophyll cells large enough so that there was room for them to have been widely separated. In 41 out of 50 cases, or 82 per cent, the two were together. This is too large a percentage to be accidental. Since many of these haustoria were located at the end of a cell three or four times as long as the haustorium, the contact between the two can have been achieved only by the motion of the nucleus to the haustorium.

Host nuclei in young and old infections and in uninfected tissue near by were measured. There is some expansion of the nucleus in infected tissue. The larger nuclei measured  $10.0\mu$  by  $7.5\mu$  in infected tissue and  $8.7\mu$  by  $7.7\mu$  in uninfected. The expansion is slight compared to that seen in infections of other rusts.

At the end of the first week of growth the mycelium has invaded an area from  $350\mu$  to  $500\mu$  in length, but not all of the intercellular spaces within that region are filled. The hyphae have not branched richly. Even at the center of the infection the fungous growth is still sparse.

Between the seventh and the ninth day the mycelium begins to spread rapidly through the leaf. This process is influenced markedly by the anatomy of the leaf.

In these sword-shaped leaves the veins run lengthwise of the leaf and parallel to one another. They are strands of vascular tissue circular in cross section and are embedded in rather dense mesophyll tissue with small intercellular spaces. From 25 to 50 per cent of the veins of later leaves are reinforced by heavy-walled strengthening tissue extending from the vein to both upper and lower epidermis. This tissue is practically without intercellular spaces. Each vein is marked on the upper side of the leaf by a ridge, so that this surface of the leaf is minutely corrugated. The stomata are arranged in longitudinal rows parallel to the veins. There usually is one row (occasionally two) on each slope between a ridge and its adjoining valley. The stomata are fewer on the lower surface of the leaf and are opposite those of the upper. Beneath each stoma is a large air chamber, and as the stomata form a close row (usually only one epidermal cell intervening between one stoma and the next), these rows of substomatal air chambers form chains of communicating passageways running from one end of the leaf to the other.

It is through these lanes that the fungus makes most rapid progress. Coarse, runnerlike hyphae traverse these air spaces, running as straight as the passageways in the leaf permit and following the lines of least resistance. (Pl. 7, A, *b*, *c*, *d*.) Even here, however, a runner

seldom grows more than  $100\mu$ , or  $125\mu$  at the most, without encountering a host cell. It may swerve and grow around it, but usually it stops, forms a haustorium mother cell and a haustorium (pl. 7, B, a, b, c), and continues growth by means of a branch pushed out just back of the haustorium mother cell (pl. 7, B, d, e). In young runners there are no septa other than those cutting off these short haustorium mother cells. In one case a runner was traced  $400\mu$  without a cross wall. The growing tip of a runner is dense and stains intensely. A little farther back (pl. 7, C) the contents are more open. The numerous nuclei are scattered or in groups with no obvious arrangement. The average diameter of these runners is  $9.5\mu$ . Still farther back from the growing tip (pl. 7, B) the runner, still nonseptate, shows scant content. There is a continuous central vacuole, and in the peripheral cytoplasm the nuclei are scattered or in pairs (pl. 7, B, f, g, h). Doubtless a part of the former content flowed on toward the growing tip, and some of it was utilized in the formation of lateral branches.

The rust also spreads transversely as far as continuous air passages in the mesophyll tissue permit. Here and there a runner gives off a small lateral branch which threads its way into the denser tissue, ramifying further and producing numerous haustoria. These feeding hyphae are variable in size, averaging  $4.7\mu$  in diameter. Many of the small intercellular spaces in the tissue around a vein are blind alleys, but some of them form continuous crooked passageways through which hyphae pass, and if the mycelium succeeds in reaching the more open tissue beyond the vein, a new set of runners is initiated, running parallel to the first. But the transverse spread of the mycelium is slow at best and is stopped on either side when a reinforced vein is reached, for the thick-walled strengthening tissue without intercellular spaces forms an impassable barrier.

So, while the transverse spread of the rust is checked when a reinforced vein is encountered, there is no mechanical barrier to the longitudinal growth, for the chains of substomatal passageways extend from one end of the leaf to the other. The result is a narrow band-shaped infection which can, and under favorable conditions does, extend the full length of the leaf.

When an infection is about 10 days old, a few septa form in the oldest part of the mycelium. These increase in number. As the mycelium extends longitudinally, the central septate area increases proportionately in size. In any older infection, all stages can be found between the nonseptate marginal region and the fully septate central area.

Some of the steps in this process have been observed. In Plate 7, D, is drawn a branching hypha in an early stage of septation. Septa,

#### EXPLANATORY LEGEND FOR PLATE 7

A.—From a 17-day infection of Jenkin club wheat. A longitudinal section showing partly septate runners, b, c, d, f, and finer mycelium in smaller air spaces, g, h, i. Subepidermal spore-producing hyphae massing at a and e.  $\times 155$ .

B.—Detail from 17-day infection on Jenkin club wheat. Partly drained runner, d e, in substomatal air space. No septa except those delimiting the haustorium mother cells a, b, c. Nuclei paired at f, g, and h.  $\times 730$ .

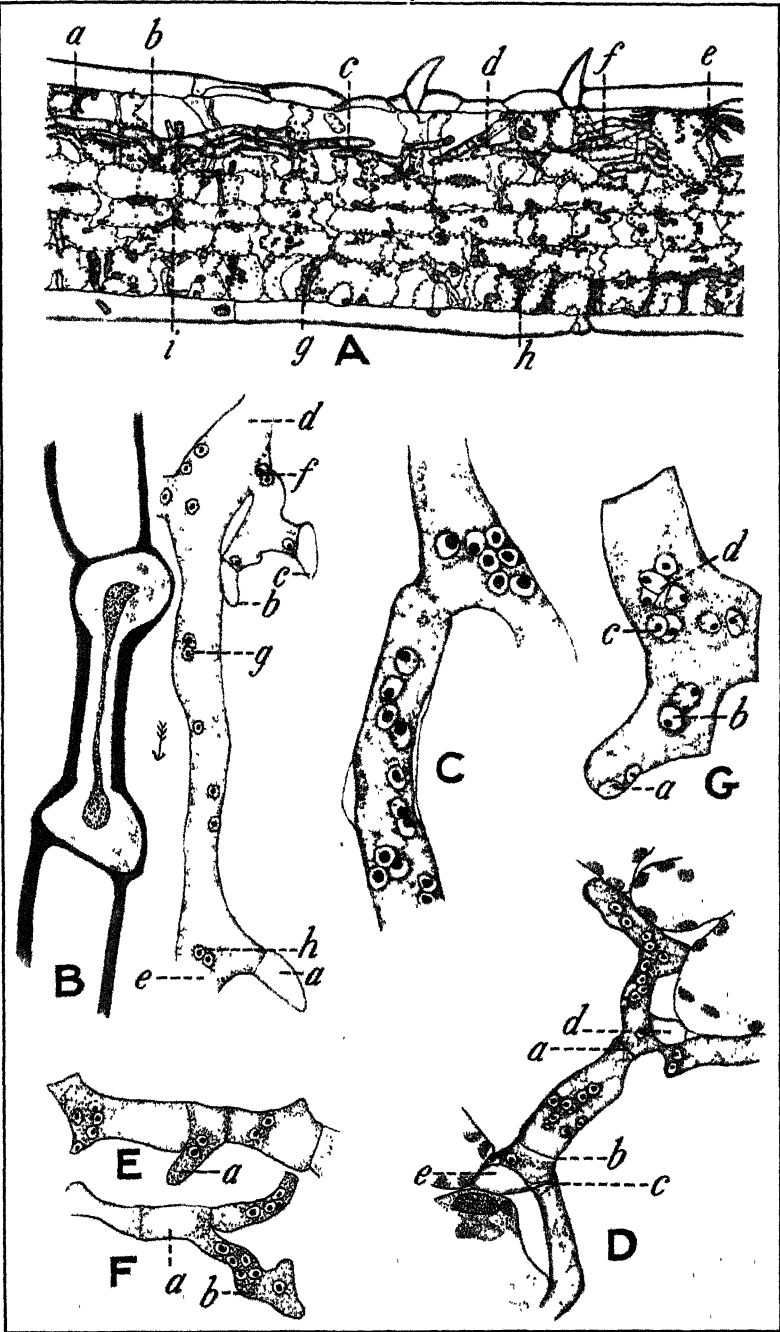
C.—Portion of nonseptate runner with rich cytoplasm and numerous irregularly arranged nuclei.  $\times 1130$ .

D.—From 16-day infection on *Bromus marginatus*. Early stage of septation. Septa at a, b, c. Cells of hyphae still multinucleate. Empty haustorium mother cells at d and e.  $\times 730$ .

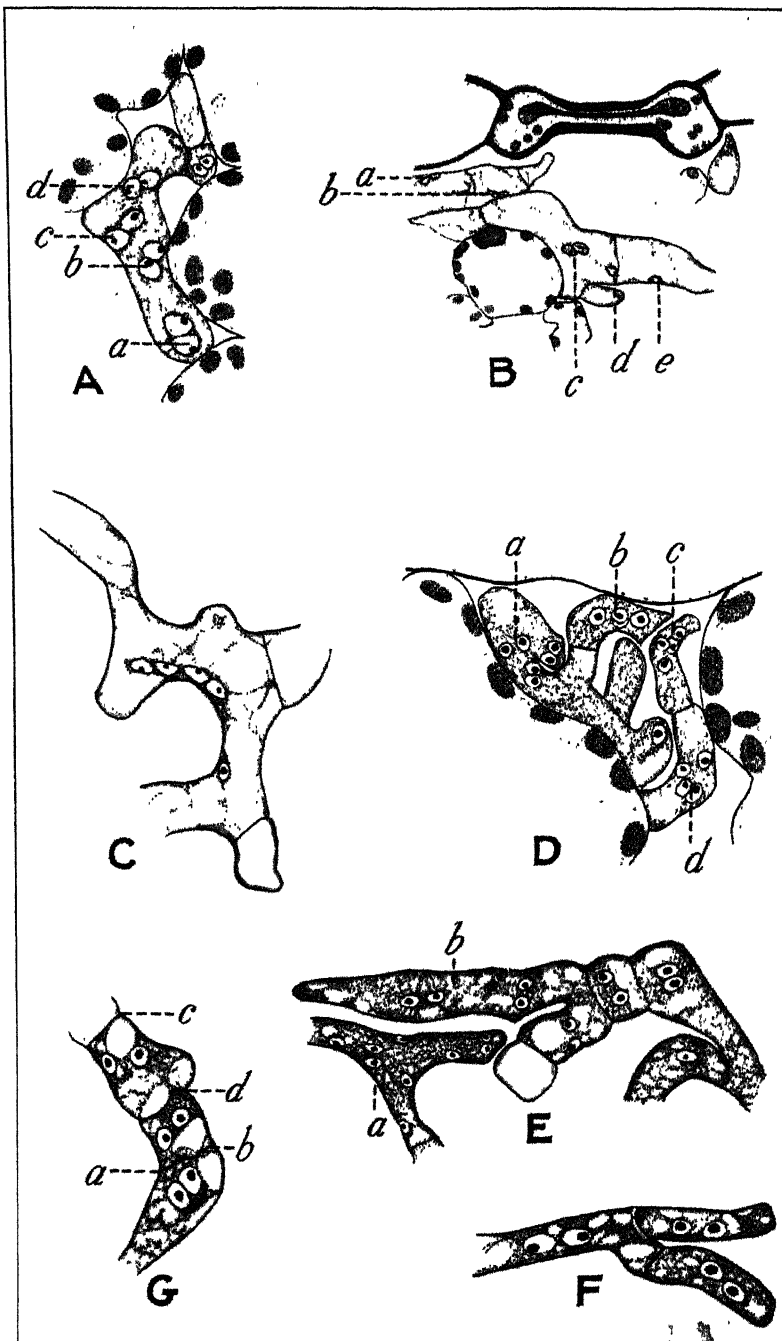
E.—As in D. Showing passage of nuclei and cytoplasm of cell into the branch, a.  $\times 730$ .

F.—As in D. The cell, a, nearly evacuated, its protoplasm having passed out into the branch, b.  $\times 730$ .

G.—From 17-day infection on Jenkin club wheat. Detail showing paired nuclei at a, b, c, and larger group of nuclei at d.  $\times 1130$ .



(For explanatory legend, see page 500)



(For explanatory legend, see page 501)

of course, delimit the two empty haustorium mother cells, *d* and *e*, but in addition there are other septa, *a*, *b*, and *c*, cutting the main hypha into short lengths. These shorter cells are still multinucleate. The cell, *bc*, appears to be small and binucleate, but really extends on behind the mother cell, *e*. The nuclei are irregularly disposed in the cells, and the number in a cell varies.

These partly septate hyphae branch freely. In Plate 7, E, a binucleate cell has pushed out a short branch, *a*, into which both cytoplasm and nuclei are passing. In Plate 7, F, the cell, *a*, is nearly empty, the cytoplasm and six nuclei having moved out into the branch *b*. Sometimes a septum near the base of the branch cuts off retreat, and the evacuated portion of the parent hypha remains quite empty.

Septation progresses unevenly, varying within a small area. Alongside of hyphae with numerous irregularly disposed nuclei (pl. 7, C and D) are others in which some or all of the nuclei are arranged in pairs. In Plate 7, G, are three pairs of nuclei *a*, *b*, *c*, and an unsorted group, *d*. In Plate 8, A, is a hypha with four pairs of nuclei, *a*, *b*, *c*, *d*, and adjoining it a small cell which may be part of the same hypha, containing a fifth pair. In this case the two nuclei of a pair are close together, or even in contact, and the pairs tend to become scattered along the length of the hypha. Sometimes the pairing of nuclei is less obvious. Of course it is possible that these pairs are determined at an earlier stage, but repeated efforts to discover this have failed. Runners as well as the smaller hyphae become septate. Perhaps the pairing at *f*, *g*, and *h* in Plate 7, B, is a beginning of this process.

This rearrangement of nuclei normally is followed by the building of septa between one pair and the next, giving rise to binucleate cells. It does not work out perfectly, for an occasional cell, as in the runner figured in Plate 8, B, may have one pair, *c*, and an extra nucleus, *d*, while the adjoining cell has only one nucleus, *e*. Septation probably remains incomplete in places. In Plate 8, C, is drawn a hypha whose contents have been drained. The five nuclei are reduced in size, and the cytoplasm consists of a few delicate strands. Hyphae near by are septate, but it is doubtful whether the depleted living contents of this cell suffice for septum building.

The appearance of septa marks the beginning of reproductive activities. So regular is the sequence of septation and reproduction that the formation of septa in any part of an infection may be considered the first step in preparation for spore formation at that point.

#### DEVELOPMENT OF UREDINIA

Beginning at the center where the mycelium is oldest and best established, the wave of septation advances along the lines of growth,

#### EXPLANATORY LEGEND FOR PLATE 8

A.—From 17-day infection on Jenkin club wheat. Detail showing paired nuclei at *a*, *b*, *c*, *d*, and *e*. × 1130.

B.—As in A. Septate runners. Binucleate cells usual as at *a* and *b*. The cell, *c*, contains a pair at *c*, and an extra nucleus at *d*, while the cell, *e*, is uninucleate. × 666.

C.—From 14-day infection on *Bromus marginatus*. Drained hypha with imperfect septation. × 1130.

D.—As in C. Subepidermal mycelium which will give rise to spores. Cells *a*, *b*, *c*, *d* are still multinucleate but the nuclei are often paired. × 1130.

E.—As in C. Young spore-producing mycelium beneath epidermis. Multinucleate cell at *a*. Nearly binucleate hypha at *b*. × 1130.

F.—As in C. Binucleate subepidermal hypha. × 1130.

G.—As in C. Binucleate hypha with septum forming at *a*, *b*, and completed septa at *c* and *d*. × 1130.

keeping always some distance behind the growing tips of the runners, and a wave of spore formation follows close behind. At regular intervals along the lines of runners, successive reproductive centers appear, each of which will develop into a uredinium. The long chains of uredinia so formed give to stripe rust its name. The units of this chain are small and definitely limited in growth. There are about 14 uredinia to a centimeter, on an average. Beyond the open uredinia in each chain is a series of five or six similarly spaced and progressively younger reproductive centers, and beyond these in turn are the isolated runners forming the vanguard.

Part of a longitudinal section of a leaf extending from the edge of one young reproductive center to the next is drawn in Plate 7, A. Runners (pl. 7, A, *b, c, d*) as well as the smaller feeding hyphae (pl. 7, A, *i, g, h*) are partly septate. At *a* and at *e* a few small hyphae with dense contents are massing below the upper epidermis in preparation for spore formation. Septation is a little more advanced at these centers than in the area between. This is noticeable at *f*, where the runners are most closely septate. Both runners and the smaller feeding hyphae contribute branches to the subepidermal growth.

The steps in the development of uredinium have been followed in detail. The first hyphae to reach the epidermis are still composed of multinucleate cells. In Plate 8, D, representing a triangular subepidermal air space, the hyphal cells, *b, c, d*, have four nuclei each, and the cell, *a*, has six near the tip and one farther back.

This condition soon changes, and alongside of a hypha with many nuclei in a cell (pl. 8, E, *a*) another may be found (pl. 8, E, *b*) in which, although growth and nuclear division are somewhat in advance of septum formation, the binucleate tendency is evident. Soon after this, hyphae with strictly binucleate cells (pl. 8, F) are found skirting along below the epidermis.

Little has been learned as to the way in which septa form. In Plate 8, G, the irregular surface (seen as a sinuous line in edge view) extending from *a* toward *b* may be a partly formed septum. Fully formed septa are plane (pl. 8, G, *c*) or slightly arched due to turgor differences on the two sides (pl. 8, G, *d*).

The amount of subepidermal growth preceding spore formation varies somewhat, but it suffices to pry the epidermis loose from the underlying mesophyll. Short, broad cells are formed with two nuclei and dense cytoplasm. (Pl. 9, A, *a, b, c, d*.) These basal cells form an almost continuous subepidermal layer, first evident at the center of (and later throughout) the little reproductive area.

#### EXPLANATORY LEGEND FOR PLATE 9

A.—From 14-day infection on *Bromus marginatus*. Binucleate spore-producing mycelium. Basal cells at *a, b, c, d*. × 1130.

B.—From infection on White Federation wheat (C. I. 4981). Young spore mother cells, *a, b*, at the edge of a uredinium. × 1130.

C.—As in B. Developing urediniospore, *b*, with two nuclei and dense cytoplasm. Stalk cell at *a*. × 1130.

D.—As in B. Growing spores, *a, b*, still thin walled. × 1130.

E.—As in B. Longitudinal section through center of uredinium, showing spores, *a*, on the upper surface, basal cells and runners, *b, c*, beneath, and the mesophyll tissue with intercellular mycelium and occasional dead cell, *d*. × 158.

F.—As in B. Longitudinal section showing the bridge of paraphyses, *a, b*, extending from one uredinium to the next. × 158.

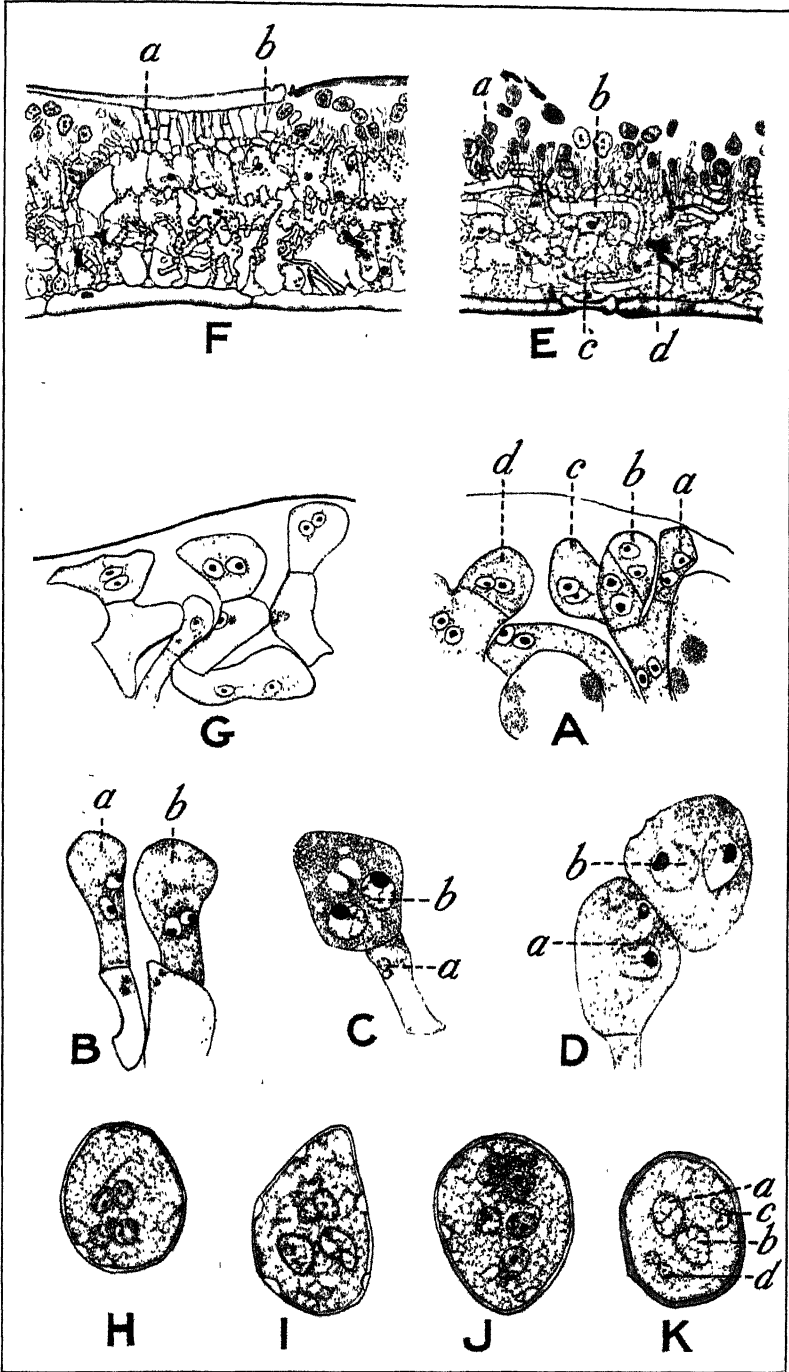
G.—From 14-day infection on *Bromus marginatus*, showing an early stage in the development of the paraphyses. × 1130.

H.—From infection on wheat C. I. 7555-1. Urediniospore with four nuclei. × 1130.

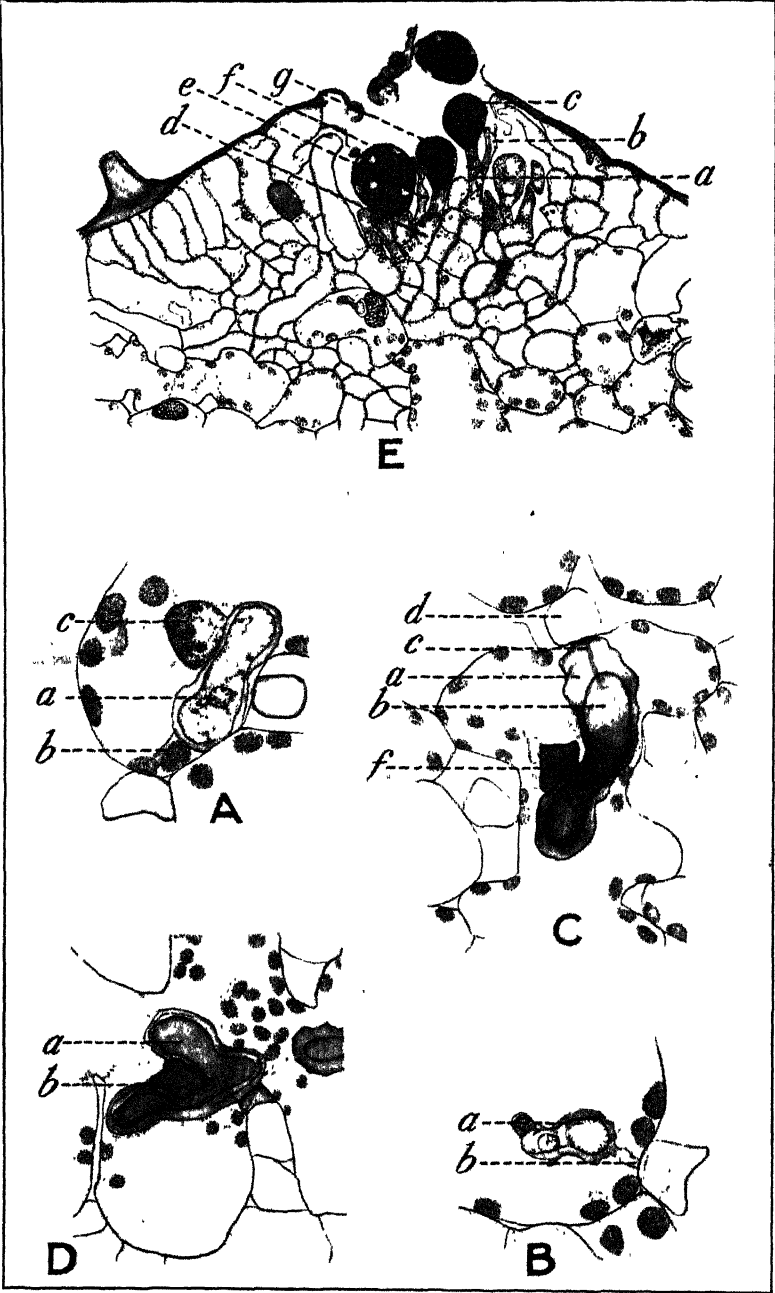
I.—As in H. Number of nuclei doubtful—probably four. × 1130.

J.—As in H. Multinucleate spore—probably six nuclei. × 1130.

K.—As in H. Spore with two nuclei, *a, b*, and two smaller irregular bodies, *c, d*. × 1130.



(For explanatory legend, see page 502)



(For explanatory legend, see page 503)



From these the spore mother cells push up, forcing up the epidermis as they grow. (Pl. 9, B, *a*, *b*.) The spore mother cell divides into stalk cell and spore, each with two nuclei. (Pl. 9, C, *a*, *b*.) The young spore grows rapidly (pl. 9, D), and since it remains thin walled until nearly mature, the details of its contents may be seen clearly. The relative position of the two large nuclei varies. They may be in line with the longitudinal axis of the spore (pl. 9, D, *a*), at right angles to this (pl. 9, D, *b*), or in any intermediate position. The very young spores, especially if subject to lateral compression, usually have the nuclei in a longitudinal row. The mature spore, already described (pl. 1, D), usually contains dense cytoplasm and two nuclei and is covered by a wall provided with numerous scattered germ pores.

Something of the appearance of a spore-bearing region at the height of its activity may be seen in Plate 9, E, representing part of a longitudinal section through the center of an open uredinium. Runners (pl. 9, E, *a*, *b*, *c*) are still conspicuous, and smaller hyphae fill the lesser air spaces of the leaf.

The cells of the mycelium appear quite empty, the contents having drained into the spore-bearing layer. The first part of this translocation of materials may have occurred as a direct flow of cytoplasm and nuclei as such into branches growing to the surface. (Pl. 7, E, F.) Much of it, however, occurred after septation was completed and could have been achieved only by a process similar to that described in other rusts (2, 3), in which the protoplasm of the hypha appears to break down into a simpler, more soluble form which can diffuse through septa. During this process the cytoplasm first decreases in quantity, and later even the nuclei become smaller and disappear, leaving the cells of the hypha free of stainable content. Haustoria as well as hyphae become drained, and in normal old age a haustorium (pl. 6, I) is nearly empty, containing but a few delicate strands of cytoplasm and the single reduced nucleus (pl. 6, I, *a*).

Not all stripe-rust haustoria undergo normal drainage. Among the haustoria beneath uredinia are some which have degenerated and died apparently without drainage. Plate 10, A and B, shows the beginnings of this change. Within each haustorium, *a*, a nucleus and cytoplasm can still be seen, but each haustorium is incased in a heavy sheath, and its neck (pl. 10, A, *b*, and B, *b*) has become modified. Later (pl. 10, C) the nucleus and cytoplasm of the haustorium break down into a mass that stains heavily and uniformly. The outline of the body, *b*, of the haustorium can still be discerned within the heavy sheath, *a*, and the slender neck, *c*, can be seen leading from it to the empty haustorium mother cell, *d*, outside of the host cell. The dead host nucleus, *f*, lies in contact with the haustorium. The host cell is not collapsed nor disordered and still contains plastids

#### EXPLANATORY LEGEND FOR PLATE 10

A.—Early stage of degeneration of haustorium from infection in leaf sheath of heading plant of wheat, C. I. 7555-1. The haustorium, *a*, is living, but it is coated with a heavy sheath, and its neck, *b*, is modified. The host cell is living and the host nucleus, *c*, lies against the haustorium. × 1130.

B.—Similar to A. The haustorium, *a*, and its neck, *b*, are heavily sheathed. × 1130.

C.—Dead haustorium from an infection on the leaf blade of heading plants of White Federation wheat (C. I. 4981). Within the heavy sheath, *a*, can be seen the haustorium, *b*, with its neck, *c*, leading to the haustorium mother cell, *d*. The dead host nucleus, *f*, lies against the haustorium. × 1130.

D.—Similar to C. The haustorium, *a*, is branched and the host nucleus, *b*, lies at the forking. × 1130.

E.—Portion of cross section of glume of wheat, C. I. 7555-1, showing small uredinium. The basal cell, *a*, gave rise to two spores, *b* (freed) and *c*. The basal cell, *d*, formed three spores, *e* (set free), *f*, and *g*. × 510.

and cytoplasm. Plate 10, D, *a*, shows a degenerate branched haustorium with the host nucleus, *b*, between the branches. Plate 11, A, is still further degenerated and not even the neck is distinguishable. These degenerate haustoria occur in old infections on all hosts studied, even though the rust has made good growth. In one advanced infection on old leaf-sheath tissue of wheat (C. I. No. 7555-1) 50 per cent of the haustoria showed this type of breakdown.

The spores are borne chiefly on the upper surface (pl. 9, E) and are at all stages of development. Just beneath them are the short broad basal cells from which they arise. The first-formed spores have been freed, leaving the withered spore stalks behind them. The younger spores must force their way up between the stalks of older spores, often under considerable pressure.

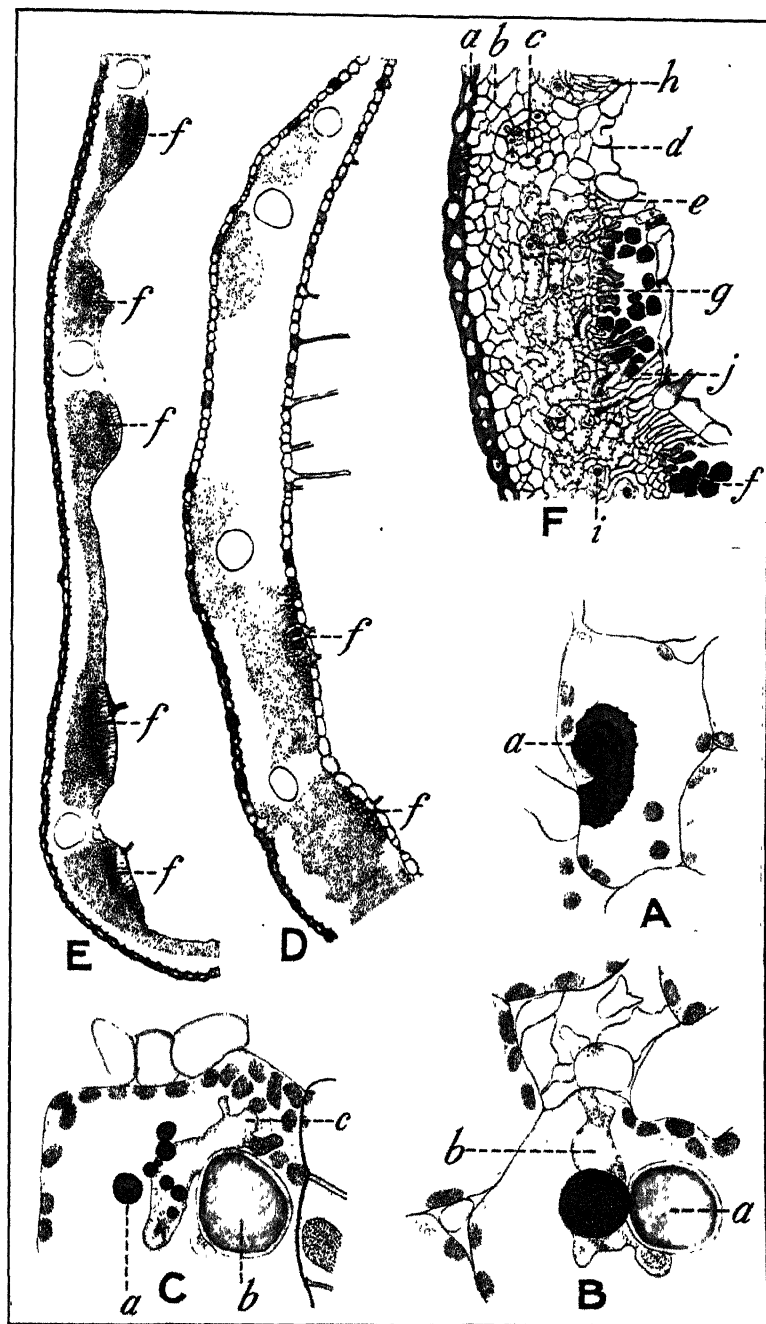
The same basal cell can give rise to several spores in succession. This shows clearly in Plate 10, E, a cross section through a small uredinium growing on a glume of wheat. The basal cell, *a*, first formed a spore at *b* (of which only the withered stalk is left) and then a second, *c*. The basal cell, *d*, formed three spores, one at *e* (evidenced by the stalk), a second at *f*, now mature, and a third, at *g*.

About the margin of the uredinium is more or less of sterile fungous tissue composed of coarse upright cells set side by side in the fashion of a palisade. These paraphyses vary greatly in number. In some infections, particularly on seedling hosts, they are practically absent. In others, each uredinium is completely girt about with sterile tissue. There is an abundance of it on either side of the small uredinium in Plate 10, E. In some chains of uredinia on nearly mature host plants there is a bridge of sterile tissue between each uredinium and the next. (Pl. 9, F, *a* to *b*.) These upright sterile cells originate in a line of basal cells similar to those at the base of the spores. When very young, this marginal sterile tissue (pl. 9, G) resembles closely in structure the young spore-bearing tissue adjoining it. The sterile tissue can be distinguished at a very early stage, however, by the vacuolate cytoplasm of its cells. The watery contents of these cells contrast sharply with the dense contents of fertile tissue at a corresponding age. (Cf. pl. 9, A and G.)

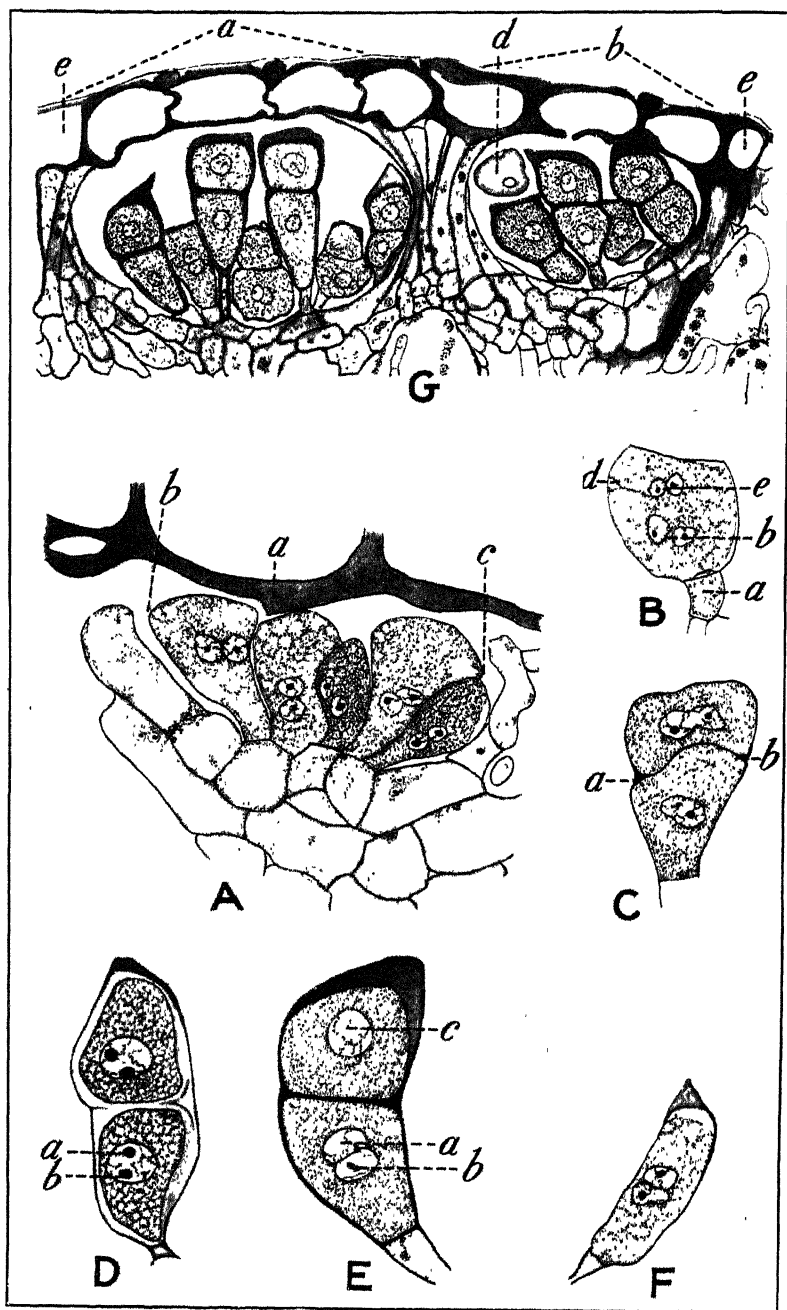
In one lot of material (nearly mature wheat plants fixed at Davis, Calif., in May, 1926) a small percentage of the last generation of urediniospores—those contemporaneous with the teliospores—contain more than two nuclei. Plate 9, H and I, shows spores with four nuclei. In Plate 9, J, the number is doubtful, but it may be more than four. In some cases two large central nuclei are found (pl. 9, K, *a* and *b*) and in addition smaller irregular bodies (K, *c*, *d*) similarly stained. These are unexplained. The percentage of these multinucleate spores is always small. When, during the following year

#### EXPLANATORY LEGEND FOR PLATE 11

- A.—From 17-day infection on Jenkin club wheat. Dead haustorium, *a*.  $\times 1130$ .  
 B.—From infection in leaf sheath of *T. vulgare* C. I. 7555-1. Green intracellular body, *a*, in living cell of host. Haustorium at *b*.  $\times 1130$ .  
 C.—From infection in leaf sheath of *T. vulgare* C. I. 7555-1. Two types of intracellular bodies—at *b* a large green body and at *a* a group of small spherical red-stained bodies.  $\times 1130$ .  
 D.—Cross section of upper end of glume of C. I. 7555-1. Semidiagrammatic.  $\times 79$ .  
 E.—Cross section through lower part of same glume.  $\times 79$ .  
 In D and E, the circles represent veins, the dotted area mesophyll, and the clear area represents rather heavily-walled empty cells without intercellular spaces. Rust occurs at points marked *f*.  
 F.—Portion of cross section of glume of C. I. 7555-1 showing outer epidermis at *a*, lined by strengthening tissue, *b*, which extends around vein, *c*, to inner epidermis at *d*. Mesophyll tissue containing uredinia at *f*, *g*, and *i*, flanked by paraphyses at *e* and *j*. Edge of next patch of mesophyll and fungus at *h*.  $\times 158$ .



(For explanatory legend, see page 501)



(For explanatory legend, see page 505)

(June, 1927), telia of stripe rust appeared on plants of *Bromus marginatus* in Berkeley, Calif., material of telia and adjoining uredinia was fixed. Urediniospores here, so far as observed, are uniformly binucleate.

The host tissues underlying uredinia (pl. 9, E, F) are generally living but impoverished. There is less evidence of the stimulation of invaded cells than in the other wheat rusts studied (2, 3). Host nuclei expand but little and die prematurely. In one infection the larger nuclei in cells containing haustoria averaged  $10\mu$  by  $7.5\mu$  as compared with  $8.5\mu$  by  $7.7\mu$  in the adjoining uninfected tissue. Plastids decrease in size, and in no case has excess starch been noted. Occasionally a mesophyll cell collapses into a little irregular red-staining mass. (Pl. 9, E, d.) These dead collapsed cells increase in number with the age of the uredinium. There may be 10 or 15 per cent of them beneath older uredinia.

In one lot of material taken from leaf sheaths of nearly mature wheat (C. I. No. 7555-1), two types of intracellular bodies were found. One (pl. 11, B, a) is spherical, ranges in size up to  $12\mu$ , stains green in the triple stain, is nearly transparent, and is homogeneous in content save for a paler outer layer. It may have any position in the cell. These green-staining spheres are abundant in the infected tissue but also have been seen in a few cells just beyond the margin of the mycelium. Since tissue more remote from the infection was not fixed, it is not certain that these bodies are due to the activities of the rust.

The other type of intracellular body is small, spherical, and stains a deep red. In Plate 11, C, both types are to be found in the same cell, the green-stained body at *b* and a group of little red-stained spheres at *a*. Both types are also to be seen in Plate 6, H, already referred to. These red globules have, so far, not been seen outside of infections. They are most numerous in cells with little protoplasm, and their formation is apparently associated with the breakdown of living matter under the influence of the fungus.

*Puccinia glumarum*, as already mentioned, can grow and reproduce in the glumes. Uredinia occur in rows running parallel to the veins of glumes and even of awns. In the glumes, nearly all of these uredinia open upon the inner face, so that the spores are shed into the narrow crevice between the glume and the inner flower parts, often lodging and accumulating there. This is a disadvantage to the rust, as such spores are not readily caught and dispersed by the wind.

The distribution of stripe rust in glumes is limited by three facts: The fungus can enter only through stomata; it can spread only through intercellular spaces; and it can form haustoria only in relatively thin-walled living mesophyll tissue and epidermis. In the

#### EXPLANATORY LEGEND FOR PLATE 12

A.—Part of a section of a leaf sheath of wheat C. I. 7555-1, representing a young telium, with spore initials, *b c*, with large nuclei and dense cytoplasm. Below are the basal cells, at the sides are young paraphyses and above is the epidermis, *a*.  $\times 1130$ .

B.—A young teliospore (on C. I. 7555-1) with stalk cell, *a*, and distal cell containing two pairs of nuclei, *b c*. A septum which will divide it into two cells is forming at *d*.  $\times 1130$ .

C.—Growing teliospore from C. I. 7555-1. Nuclear fusion is in progress. The wall is still thin except at ends of septum, *a b*.  $\times 1130$ .

D.—Nearly mature teliospore (on C. I. 7555-1) with dense cytoplasm and heavy walls. The fusion nucleus of each cell still contains two nucleoles, *a, b*.  $\times 1130$ .

E.—Mature teliospore (on C. I. 7555-1) showing delayed nuclear fusion in one cell, *a b*, and completed fusion in the other, *c*.  $\times 1130$ .

F.—Single-celled teliospore (on C. I. 7555-1) at edge of sorus.  $\times 1130$ .

G.—Section (of C. I. 7555-1) through two mature telial units, *a, b*, each containing a compact group of spores springing from a pseudoparenchymatous base of fungous cells, inclosed laterally by upright, somewhat compressed paraphyses, and roofed by the epidermis of the host, *e e*. Urediniospore at *d*.

wheat glumes examined (C. I. No. 7555-1) much of the interior is made up of strengthening tissue composed of empty, rather thick-walled cells without intercellular spaces. The fungus can not penetrate this tissue.

Plate 11, D, represents semidiagrammatically part of a cross section through the upper end of a glume and Plate 11, E, one from the lower third of a glume. In both, the circles represent veins, the dotted areas mesophyll, and the clear areas correspond to the strengthening or mechanical tissue. In the lower part of the glume (pl. 11, E) the strengthening tissue surrounds the veins and forms a continuous sheet lining the outer (convex) epidermis. The mesophyll faces the inner epidermis between veins and is thickest near the veins. In the upper part of the glumes the arrangement is more irregular. Part of the mesophyll fronts outward. The outer epidermis has thick walls throughout. The inner epidermis is thinner walled—markedly so in the lower, less-exposed part. So far as noted, stomata occur only where the epidermis is in contact with mesophyll.

So the rust (points marked *f* in the diagrams) enters only where there is mesophyll, spreads only through its spaces, and grows out to the inner surface (the only one accessible except at the tip of the glume) to form uredinia.

The tissues of the glume are drawn at higher magnification in Plate 11, F. At *a* is the heavy-walled outer epidermis, and lining it is a sheet of strengthening tissue, four or five cells thick, free of fungus. This tissue extends around the vein, *c*, and on to the inner epidermis at *d*. Under the inner epidermis from *e* to *f* is mesophyll permeated by fungus. There is one small uredinium at *g* and the edge of a second at *f*. There is a bridge of paraphyses between the two. At *h*, above the vein, are paraphyses adjoining a third uredinium. Plate 10, E, already described, is also from a glume.

#### DEVELOPMENT OF TELIA

Telia are formed on host plants nearing maturity. They occur on the leaf sheaths, on the glumes, and, more rarely, on the leaf blades. A brief study has been made of telia on the leaf sheath, where they are most abundant. The small compact telia form along the edges of old uredinia. Exceptionally, the two kinds of spores may be found mixed in the same sorus.

A typical young telium is drawn in Plate 12, A. The cells that are to become spores, *b* to *c*, are easily distinguished by their dense contents and large nuclei. Their form varies with the available space. They may be misshapen by pressure against the heavy epidermis. When relatively free, as in this case, they are balloon shaped.

In development, the initial cell first divides, forming a short stalk cell. (Pl. 12, B, *a*.) Then the two nuclei in the spore proper undergo division, forming four nuclei. (Pl. 12, B, *b*, *c*.) In this case the septum which will divide the spore into two cells is beginning to form at *d*.

As the spore grows and takes shape, the two nuclei in each cell fuse. This process may begin as soon as the spore is two celled and in some cases is not complete until the spore is nearly mature. In Plate 12, C, a half-grown spore, still thin walled, shows an early stage of fusion. In each cell the two nuclei have come in contact, and the nuclear membrane at the surface of contact has disappeared. The two sets of chromatin still appear to be distinct. Later the fusion nucleus

becomes round but may still show the nature of its origin by the presence of the two nucleoles. (Pl. 12, D, *a*, *b*.)

Slight irregularities occur. Sometimes fusion is delayed in one cell (pl. 12, E, *a*, *b*), although completed in the other (pl. 12, E, *c*). Occasionally one finds a one-celled teliospore (pl. 12, F) and more rarely a three-celled one.

Something of the appearance of the mature telia is shown in Plate 12, G. Each of the two compact telial units, *b* and *a*, is roofed by the heavy epidermis of the host, *e*, and walled in at the sides by a palisade of paraphyses similar to that surrounding the uredinia. The spores are packed closely together and shaped to some extent by the available space. At *d* is part of a urediniospore. Farther on in this sorus urediniospores predominate.

### DISCUSSION

The earliest characteristic difference found between stripe rust and other wheat rusts is the absence of a well-marked appressorium. This was also noted by Pole Evans (21). The contents of the germ tube are somewhat denser at the growing tip, but the tube swells little, if at all, on reaching the stoma, and ordinarily there is no septum marking off a separate appressorial cell. Even before entering the host the tendency in stripe rust toward the minimum of cross walls finds expression. This procedure would appear to be a disadvantage to the rust, for in cases of delayed entry a thin-walled germ tube exposed to wind and sun would probably stand a poorer chance of survival than the compact appressorial cushion of other rusts.

The incubation period in stripe rust of wheat is from two to four days longer than in either of the other wheat rusts. Urediniospores germinate overnight, yet inoculated leaves do not "fleck" until the eighth, ninth, or even tenth day (varying somewhat with the host and the time of year). A part of this delay is due to the prolonged juvenile period of the rust. On the second day the fungus consists of a small substomatal vesicle and one to three short slender infecting hyphae, each of which has formed a little haustorium. During the next two days the only development is a swelling of the vesicle and initial hyphae to several times their original dimensions. Since these are only 1 or 2 or, at most, 3 little haustoria to draw food from the host, this process is necessarily slow.

Rice (35) finds in early infection stages of corn rust a well-developed intercellular mycelium before the development of haustoria, and concludes that the fungus is not entirely dependent on the haustorium for its nutrition. In stripe rust, on the contrary, the conspicuous bulging of the first infecting hyphae just back of the haustorium mother cells gives ample evidence that the food is coming in through these mother cells from the haustoria.

No evidence of direct feeding by the hyphae has been noted in wheat rusts. Haustoria are formed at the beginning of infection and increase in number in direct proportion to the development of the mycelium. They seem adequate for the task of absorbing food for the parasite, but, of course, it would be difficult either to prove or disprove that a small amount of food was absorbed directly by the hyphae. If, in some rusts, a part of the food is absorbed directly by the hyphae without the formation of the special absorbing organs,

the haustoria, the hitherto baffling problem of growing rusts in culture media looks a little less impossible of solution.

It is of interest that the exceptional size of stripe-rust hyphae is not attained gradually as the fungus grows out into the tissue and establishes more contacts with the host, but is initiated right at the outset of the vegetative life. The first hypha pushed out from the expanded fungous mass at the stoma of entry is full sized and is multinucleate and nonseptate like the later hyphae.

Rice (35) and the writer (3) compiled from rust literature data on the number of nuclei in haustoria of gametophytic and sporophytic mycelia. The numbers as reported are somewhat variable, but uninucleate haustoria predominate in the gametophyte and binucleate in the sporophyte. Wheat rusts do not conform to this. *Puccinia graminis* (2), *P. triticea* (3), and *P. glumarum* have regularly uninucleate haustoria in the uredinal (sporophytic) generation. In *P. glumarum* only two haustoria have been seen which had two nuclei, and none with more. The haustorium mother cells have a variable nuclear content, but apparently all but one of these nuclei degenerate before the haustorium is formed.

The restrictions in the spread of stripe-rust mycelia imposed by host tissues impenetrable to the fungus were fully described by Eriksson and Henning in "Getreideroste" (20). Hursh (26), in his study of the relation of morphologic structure of the wheat plant to resistance to *Puccinia graminis tritici*, found a similar effect on the progress and ultimate development of the invading fungus. These limitations, together with the production by stripe rust of vigorous runners which find their freest path in the open spaces beneath the rows of stomata, determine the course of progress and the final form of the infection.

The active period of any one portion of an infection is brief. By the time the host tissues beneath one uredinium are impoverished, new uredinia down the line are shedding spores. The impoverishment of infected host tissues takes place somewhat faster in stripe rust than in stem and leaf rusts of wheat. Moreover, degenerate haustoria, hitherto regarded (2) as an indication of uncongenial relations between host and rust, are found in older parts of infections on all the hosts studied.

Perhaps the more rapid breakdown of relations between host and pathogene in any given area is to be correlated with the unusual ability of the latter to move on to fresh areas. At any rate, this continued migration gives added efficiency and increased spore production.

In stripe rust there is associated with an ability to form an indefinite series of uredinia a definite limitation in the size of the individual uredinium. The uredinia of many rusts spread marginally, forming new spore-bearing cells around the edges as the central part nears exhaustion. In stripe rust the definite little spore-bearing area usually is bordered by sterile cells which are probably homologous to spores (or spore mother cells when the final septum is omitted) and whose appearance suggests a half-eradicated tendency toward the marginal growth of the uredinium common in many rusts. The function of these paraphyses is doubtful. They may perhaps be of some service as buffer tissue, helping to raise the epidermis and to release the young spores from its pressure. It should be mentioned, however,



that uredinia lacking this sterile tissue—and such are not infrequent—show no lack of effectiveness.

On one of the hosts studied, intracellular bodies of two types are found in infected areas. Beauverie (8) describes several types of intracellular bodies found in degenerating plant tissues, some of which are formed in cells containing fungous haustoria. He suggests that the “nucleoles” which constituted for Eriksson the histologic proof of mycoplasma are nothing but intracellular bodies of one or more types.

Eriksson's figures (17) show some of the “Plasmanucleoli” (supposed mycoplasma) as little red-stained globules lying free in the cell and others as connected with the fungus outside by a fine tube extending to and through the host cell wall. The free globules were probably red-stained intracellular bodies of the sort figured in this paper (pl. 11, C), and the others were undoubtedly early stages in the formation of haustoria. The spherical heads of very young haustoria stain deep red with the triple stain.

Another type of intracellular body was figured by Ruttle and Fraser (36) in infections of *Puccinia coronata* Cda., and judging by the figures, this same type was seen by Zach (39) in infections of *P. glumarum*.

Prior to septation, the nuclei are irregularly disposed, isolated or in groups, along the length of the hyphae. When the time for septation comes, these nuclei sort themselves into pairs which become fairly uniformly distributed. The basis of this assortment is unknown, but it is possible that there is here a pairing of “plus” and “minus” elements such as is known in Phycomycetes, Ascomycetes, Ustilaginales, and Agaricales, and is now announced by Craigie (13) in one of the rusts; and that somehow the pairs of nuclei in stripe rust are comparable to the pairs in binucleate mycelium of other rusts. When one considers, however, that no aecial stage, and hence no fusion of uninucleate cells, is known in stripe rust, the mechanism of this process becomes obscure. Of course, even if (as is not proved) stripe rust lost its aecial stage long ago, whatever basis of assortment originally obtained might still persist.

When the binucleate condition is once established, further development parallels that of other rusts. The new cells formed agree closely in size, form, and activities with the corresponding stages in rusts with regularly binucleate mycelium, and binucleate spores are formed in the usual fashion.

In uredinia adjoining telia in one lot of material, a few of the urediniospores contain more than two nuclei. It is doubtful what weight to give this. These multinucleate spores have been seen so far only in association with telia, and this aroused a suspicion that stripe rust, whose aecial host is yet to be found, may be executing some short cut in its life history by which a small percentage of spores in the final uredinial generation on a maturing host function as teliospores. On this basis, the young urediniospore should contain two nuclei, then one, then two again, and finally four. Evidence on this point was not available, for the uredinia adjoining the telia in this material were old, and very few young urediniospores were present. During the next year, when an attempt was made to secure a slightly earlier stage, only binucleate urediniospores were found.

On the other hand, stripe rust has multinucleate cells through much of its history, and the presence of more than two nuclei in a urediniospore may be only one more expression of this mode of life. It is consistent with the rest of the life history.

The brief study of the development of the teliospore included here suffices to show that it is quite regular and like that of other rusts. The germination of stripe-rust teliospores and the formation of sporidia have been described by Eriksson and Henning (20). No deterioration in this part of the cycle has been observed, yet, so far as known, it is useless. The fact that teliospores and sporidia continue to be formed year after year can not be taken as proof of the existence of an aecium. Elsewhere, functionless structures have persisted, as, for example, in regularly apogamous ferns (1) which continue to form and liberate active spermatozoids. In other words, defective telia and sporidia would have constituted evidence against the presence of an aecium in the life cycle, but normal telia and sporidia do not prove its existence.

No process has been seen in the uredinial generation of stripe rust which would compensate for a missing aecial stage. Of course, among the hundreds of nuclei in the large vegetative hyphae, nuclear fusion and reduction could take place undetected, but its occurrence at that stage is improbable. With the doubtful exception of four-nucleate urediniospores, no positive evidence has been noted which would lead to a belief in the absence of an aecial stage.

#### SUMMARY

Urediniospores of *Puccinia glumarum* contain two, or rarely more, nuclei. The spore wall has 10 to 16 germ pores. On germinating, the germ tube grows to the stoma and enters without forming a definite appressorium.

The substomatal vesicle is multinucleate. It pushes out one to three short infecting hyphae, each of which forms a small haustorium. On the second, third, and fourth days the vesicle and infecting hyphae expand to several times their original volume.

From this expanded mass at the stoma of entry, a hypha grows out into the intercellular spaces of the leaf tissue. Young hyphae are coarse, multinucleate, and, apart from haustorium formation, nonseptate.

Hauustoria are formed in abundance. The haustorium mother cell is a short, broad, terminal cell of a hypha, containing two to six nuclei. The haustorium is uninucleate, simple or branched, and has a maximum length of  $35\mu$  or  $40\mu$ . The host nucleus is usually associated with the haustorium. In normal old age the haustorium becomes drained and nearly empty. Some of the haustoria of older infections on all hosts studied undergo degeneration. The necks of others become encrusted with granular, dark-stained matter.

Vigorous nonseptate runners grow lengthwise of the leaf through substomatal passageways, spreading the rust. Branches from these runners permeate the smaller air spaces of the tissues. The transverse spread is limited by leaf veins reinforced by strengthening tissue impenetrable to the fungus.

About the tenth day after inoculation, septation begins in the older parts of the mycelium, and thereafter the septate area spreads, keeping a short distance behind the advancing runners. The first

septa cut a hypha into long cells, then nuclei (hitherto irregularly disposed) become arranged in pairs distributed along its length, and further septa cut it into binucleate cells.

During septation, branches from both the runners and the feeding hyphae grow to the upper epidermis and form there subepidermal binucleate spore-bearing cells. Urediniospores are usually binucleate. A basal cell may give rise to several spores in succession. A few of the last urediniospores formed on a maturing host plant may be multinucleate. During spore formation the host tissues beneath become impoverished and the mycelium drained. Intracellular bodies of two types are found in infections on one host.

Uredinia are small and definitely limited in size and are usually bounded by a zone of paraphyses. This limitation in the size of the individual uredinium is compensated by the production of many uredinia in succession along the lines of advance of the runner. Chains of one hundred or more uredinia are not rare.

In glumes, much of the tissue is impenetrable to the hyphae. The mesophyll tissue usually faces the inner surface of the glume and the stomata occur in epidermis adjoining mesophyll tissues, so the rust usually enters from the inner surface, spreads through the mesophyll, and comes to the inner surface to form uredinia.

Telia may form as the host matures. The development of the teliospore is normal. The spore initial divides, forming a stalk cell, and divides again, forming a two-celled spore. Each cell contains two nuclei which fuse as the spore matures.

No positive evidence has been noted of any compensatory process in the uredinal generation which would replace an aecial stage.

#### LITERATURE CITED

- (1) ALLEN, R. F.  
1914. STUDIES IN SPERMATOGENESIS AND APOGAMY IN FERNS. Wis. Acad. Sci., Arts, and Letters, Trans. 17:1-56, illus.
- (2) ———  
1924. CYTOLOGICAL STUDIES OF INFECTION OF BAART, KANRED, AND MINDUM WHEATS BY PUCCINIA GRAMINIS TRITICI FORMS III AND XIX. Jour. Agr. Research 26: 571-604, illus.
- (3) ———  
1926. A CYTOLOGICAL STUDY OF PUCCINIA TRITICINA PHYSIOLOGIC FORM II ON LITTLE CLUB WHEAT. Jour. Agr. Research 33: 201-222, illus.
- (4) ARMSTRONG S. F.  
1922. THE MENDELIAN INHERITANCE OF SUSCEPTIBILITY AND RESISTANCE TO YELLOW RUST (PUCCINIA GLUMARUM, ERIKSS. ET HENN.) IN WHEAT. Jour. Agr. Sci. [England] 12: 57-96.
- (5) ARRHENIUS, O.  
1924. UNTERSUCHUNGEN ÜBER DEN ZUSAMMENHANG VON GELBROSTRESISTENZ UND DER AKTUELLEN UND POTENTIELLEN AZIDITÄT DES ZELLSAFTES UND DER GEWEBE. Ztschr. Pflanzenkrankh. 34: 97-101.
- (6) BEAUVERIE, J.  
1914. SUR L'EFFICACITÉ DES GERMES DE ROUILLES CONTENUS DANS LES SEMENCES DES GRAMINÉES POUR LA PROPAGATION DE LA MALADIE. Compt. Rend. Acad. Sci. [Paris] 158: 1196-1198.
- (7) ———  
1923. LA ROUILLE JAUNE DU BLÉ (PUCCINIA GLUMARUM) EN 1923. Compt. Rend. Acad. Sci. [Paris] 177: 969-971.
- (8) ———  
1926. SUR LES BASES CYTOLOGIQUES DE LA THÉORIE DU MYCOPLASMA. Compt. Rend. Acad. Sci. [Paris] 182: 1347.
- (9) BIFFEN, R. H.  
1905. MENDEL'S LAWS OF INHERITANCE AND WHEAT BREEDING. JOUR. Agr. Sci. [England] 1: 4-48, illus.

- (10) BIFFEN, R. H.  
1907-12. STUDIES IN THE INHERITANCE OF DISEASE RESISTANCE. *Jour. Agr. Sci. [England]* 2: 109-128, 1907; 4: 421-429, 1912.
- (11) BOLLEY, H. L.  
1898. EINIGE BEMERKUNGEN ÜBER DIE SYMBIOTISCHE MYKOPLASMATHEORIE BEI DEM GETREIDEROST. *Centbl. Bakt. [etc.]* (2) 4: 855-859, 887-896, 913-919, illus. (Abstract in *Amer. Assoc. Adv. Sci. Proc.* 47: 408.)
- (12) BROOKS, F. T.  
1921. THE INHERITANCE OF DISEASE-RESISTANCE IN PLANTS. *British Mycol. Soc. Trans.* 7: 71-78.
- (13) CRAIGIE, J. H.  
1927. EXPERIMENTS ON SEX IN RUST FUNGI. *Nature* 120: 116-117, illus.
- (14) ERIKSSON, J.  
1897. EINE ALLGEMEINE UEBERSICHT DER WICHTIGSTEN ERGEBNISSE DER SCHWEDISCHEN GETREIDEROSTUNTERSUCHUNG. *Bot. Centbl.* 72: 321-325, 354-362.
- (15) ———  
1898. PRINCIPAUX RÉSULTATS DES RECHERCHES SUR LA ROUILLE DES CÉRÉALES EXÉCUTÉES EN SUÈDE. *Rev. Gén. Bot.* 10: 33-48, illus.
- (16) ———  
1901-02. SUR L'ORIGINE ET LA PROPAGATION DE LA ROUILLE DES CÉRÉALES PAR LA SEMENCE. *Ann. Sci. Nat., Bot.* (8) 14: 1-124; (8) 15: 1-160, illus.
- (17) ———  
1904-05. ÜBER DAS VEGETATIVE LEBEN DER GETREIDEROSTPILZE, I-IV. *K. Svenska Vetensk. Akad. Handl. (n. f.)* Bd. 37, no. 6, 19 p.; Bd. 38, no. 3, 18 p.; Bd. 39, no. 5, 41 p., illus.
- (18) ———  
1905. ON THE VEGETATIVE LIFE OF SOME UREDINEAE. *Ann. Bot. [London]* 19: 55-59.
- (19) ———  
1921. THE MYCOPLASM THEORY—IS IT DISPENSABLE OR NOT? *Phytopathology* 11: 385-388.
- (20) ——— and HENNING, E.  
1896. DIE GETREIDEROSTE. 463 p., illus. Stockholm. (Meddel. K. Landbr. Akad. Exp. n:o 38.)
- (21) EVANS, I. B. POLE  
1907. THE CEREAL RUSTS. I. THE DEVELOPMENT OF THEIR UREDO MYCELIA. *Ann. Bot. [London]* 21: 441-466, illus.
- (22) HENNING, E.  
1919. ANTECKNINGAR OM GULROSTEN (*PUCCINIA GLUMARUM*). *K. Landtbr. Akad. Handl. Och. Tidskr.* 58: 401-418, illus. (Also issued as Meddel. Centralanst. Försöksv. Jordbruksområdet [Sweden], no. 192, 20 p., illus.)
- (23) HUMPHREY, H. B., HUNGERFORD, C. W., and JOHNSON, A. G.  
1925. STRIPE RUST (*PUCCINIA GLUMARUM*) OF CEREALS AND GRASSES IN THE UNITED STATES. *Jour. Agr. Research* (1924) 29: 209-227, illus.
- (24) HUNGERFORD, C. W.  
1923. STUDIES ON THE LIFE HISTORY OF STRIPE RUST, *PUCCINIA GLUMARUM* (SCHM.) ERIKSS. & HENN. *Jour. Agr. Research* 24: 607-620, illus.
- (25) ——— and OWENS, C. E.  
1923. SPECIALIZED VARIETIES OF *PUCCINIA GLUMARUM*, AND HOSTS FOR VARIETY TRITICI. *Jour. Agr. Research* 25: 363-402, illus.
- (26) HURSH, C. R.  
1924. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO *PUCCINIA GRAMINIS TRITICI* ERIKSS. AND HENN. *Jour. Agr. Research* 27: 381-412, illus.
- (27) KLEBAHN, H.  
1898. EIN BEITRAG ZUR GETREIDEROSTFRAGE. *Ztschr. Pflanzenkrank.* 8: 321-342, illus.
- (28) ———  
1900. BEITRÄGE ZUR KENNTNIS DER GETREIDEROSTE. II. *Ztschr. Pflanz-enkrank.* 10: 70-96

- (29) KLEBAHN, H.  
1904. EINIGE BEMERKUNGEN ÜBER DAS MYCEL DES GELBROSTES UND ÜBER DIE NEUESTE PHASE DER MYKOPLASMA-HYPOTHESE. Ber. Deut. Bot. Gesell. 22: 255-261, illus.
- (30) LINDFORS, T.  
1924. STUDIEN ÜBER DEN ENTWICKLUNGSVERLAUF BEI EINIGEN ROSTPILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTSPUNKTEN. Svensk. Bot. Tidskr. 18: 1-84, illus.
- (31) MARRYAT, D. C. E.  
1907. NOTES ON THE INFECTION AND HISTOLOGY OF TWO WHEATS IMMUNE TO THE ATTACKS OF PUCCINIA GLUMARUM, YELLOW RUST. Jour. Agr. Sci. [England] 2: 129-138, illus.
- (32) MASSEE, G.  
1899. THE CEREAL RUST PROBLEM—DOES ERIKSSON'S MYCOPLASMA EXIST IN NATURE? Nat. Sci. 15: 337-346.
- (33) NILSSON-EHLE, H.  
1909-11. KREUZUNGSUNTERSUCHUNGEN AN HAFER UND WEIZEN. Lunds Univ. Årsskr. (n. f.) Afd. 2, Bd. 5, nr. 2, 122 p. 1909; Bd. 7, nr. 6, 84 p., 1911.
- (34) PESOLA, V. A.  
1927. KEVÄTVEHNÄN KELTA RUOSTEENKESTÄVYYDESTÄ. Valtion Maatalouskoettomin. Julkaisu, no. 8, 176 p., illus. (With English abstract, 22 p.)
- (35) RICE, M. A.  
1927. THE HAUSTORIA OF CERTAIN RUSTS AND THE RELATION BETWEEN HOST AND PATHOGENE. Bul. Torrey Bot. Club 54: 63-153, illus.
- (36) RUTTLE, M. L., and FRASER, W. P.  
1927. A CYTOLOGICAL STUDY OF PUCCINIA CORONATA CDA. ON BANNER AND COWRA 35 OATS. Calif. Univ. Pubs., Bot. 14: 21-54, illus.
- (37) WARD, H. M.  
1903. ON THE HISTOLOGY OF UREDO DISPERSA ERIKSS., AND THE "MYCOPLASM" HYPOTHESIS. Roy. Soc. [London], Phil. Trans. (B) 1926: 29-46, illus.
- (38) ———  
1905. RECENT RESEARCHES ON THE PARASITISM OF FUNGI. Ann. Bot. [London] 19: 1-54.
- (39) ZACH, F.  
1910. CYTOLOGISCHE UNTERSUCHUNGEN AN DEN ROSTFLECKEN DES GETREIDES UND DIE MYCOPLASMATHEORIE J. ERIKSSON'S. Sitzber. Akad. Wiss. Wien, Math. Naturw. Kl. (1) 119: 307-330, illus.
- (40) ZUKAL, H.  
1899. UNTERSUCHUNGEN ÜBER DIE ROSTPILZKRANKHEITEN DES GETREIDES IN ÖSTERREICH-UNGARN (I. REIHE). Sitzber. Akad. Wiss. Wien, Math. Naturw. Kl. (1) 108: 543-562.



# THE GROWTH OF RHODE ISLAND REDS AND THE EFFECT OF FEEDING SKIM MILK ON THE CONSTANTS OF THEIR GROWTH CURVES<sup>1</sup>

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## INTRODUCTION

The purpose of the study here reported was to obtain data regarding the growth of Rhode Island Red cockerels, capons, and pullets, and to determine what effect the feeding of skim milk would have on their growth. Inasmuch as other investigators who have made similar studies have said little or nothing about the variability of their data, it was decided that the present study should include this feature.

In order to obtain the desired data it was proposed that two similar groups of chicks be used and that one group be fed a ration presumably adequate in every way for normal growth and that the other group be fed the same ration and in addition be allowed free access to skim milk at all times.

## EXPERIMENTAL PROCEDURE

Two groups of chicks were used—one of 174, which was hatched March 14, 1924, and another of 191, hatched 10 days later. Each chick in each group was weighed as soon after hatching as possible and at age intervals of one week until 18 weeks old and thereafter every other week. The feed placed before the two groups was identical from the start, except that the group hatched last had access to sour skim milk and water at all times, whereas the one hatched first was permitted to drink only water.

The first group was put into a colony brooder house, 10 by 10 feet, and allowed access to a grass yard about 60 by 75 feet. The chicks were thus reared until they were 10 weeks old, at which age the sexes were separated. Until that time the mortality was 20 chicks. Of those living, 80 were pullets and 74 were cockerels. The latter were divided as evenly as possible in respect to weight, and 40 were caponized, 3 dying following the operation. Fifty-five pullets were selected by taking every other one as they were caught, the remainder being discarded in order to reduce the pullets to more nearly the same number as the cockerels and the capons. Thus at the beginning of the eleventh week there were 34 cockerels, 37 capons, and 55 pullets. Each lot, now designated as lot 1 cockerels, lot 1 capons, and lot 1 pullets, was put in a colony brooder house, 10 by 10 feet, surrounded by a grass yard about 60 by 35 feet. The mortality

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from the beginning of the eleventh week to the end of the period the chicks were under observation was as follows:

Lot 1 cockerels (eleventh to thirty-fourth week).....	3
Lot 1 capons (eleventh to thirty-eighth week).....	2
Lot 1 pullets (eleventh to twenty-sixth week).....	2

Thus the total number in each of the lots on which growth observations were made from hatching time to the end of the experiment was: 31 cockerels, 35 capons, and 53 pullets.

The second group received the same treatment as the first group. At the end of the tenth week the mortality was 28 chicks. Of the surviving chicks 80 were pullets and 83 were cockerels. The latter, as before, were divided into two groups and 39 were caponized, two dying following the operation. Forty pullets were again selected in the manner described. There were, then, at the beginning of the eleventh week: 44 cockerels, 37 capons, and 40 pullets. These lots, from this point on, were designated as lot 2 cockerels, lot 2 capons, and lot 2 pullets. For the remainder of the period that the chicks were under observation the mortality was as follows:

Lot 2 cockerels (eleventh to thirty-fourth week).....	4
Lot 2 capons (eleventh to thirty-eighth week).....	3
Lot 2 pullets (eleventh to twenty-sixth week).....	2

Thus the total number in each of the lots on which growth observations were made from hatching time to the end of the experiment was: 40 cockerels, 34 capons, and 38 pullets.

All six lots of chicks received the same treatment and the same feed throughout, with the one exception that in addition to the solid feed fed lot 2 cockerels, lot 2 capons, and lot 2 pullets had free access at all times to sour skim milk and water, whereas lot 1 cockerels, lot 1 capons, and lot 1 pullets were allowed only water. For the first two weeks a slightly moistened mash was fed three times daily; this mash consisted of:

	Parts by weight
Corn meal.....	2
Oats.....	2
Middlings.....	1
Bran.....	1
Whole boiled egg (including shell).....	1

A commercial chick scratch feed was also fed twice daily.

After the first two weeks a dry mash was kept before the chicks in self-feeding hoppers; this mash was mixed as follows:

	Parts by weight
Corn meal.....	3
Crushed oats.....	2
Middlings.....	1
Bran.....	1
Meat scrap (60 per cent).....	1

In addition to the above mash the chick scratch feed was fed three times daily until the chicks were 4 weeks old; at that age a change was made to a scratch feed containing equal parts of coarsely cracked corn and wheat. Each lot, as previously noted, had access to grass range.

#### THE GROWTH DATA

Each chick, as before stated, was weighed every week until 18 weeks old and thereafter every other week. In order to determine the vari-



ability of the live weights of the chicks in each lot, the average live weight, the standard deviation, and the coefficient of variation were calculated. For the purpose of facilitating comparison of the average growth of each lot, the average live weights are brought together and presented in Table 1. In Table 2 the probable error of the mean (average) live weight, the standard deviation and its probable error, and the coefficient of variation and its probable error are given.

TABLE 1.—Average weight of the chicks in each lot at the ages indicated

Age (weeks)	Average weight of—					
	Cockerels		Capons *		Pullets	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
	Grams	Grams	Grams	Grams	Grams	Grams
0.....	40.40	39.82	40.86	40.73	39.69	38.99
1.....	63.95	62.99	62.88	65.85	61.87	62.64
2.....	85.78	99.22	83.88	98.26	81.37	94.00
3.....	110.27	163.33	106.57	161.84	104.02	148.60
4.....	131.84	218.33	132.27	219.80	121.89	194.06
5.....	173.96	317.62	173.77	312.79	158.04	281.58
6.....	245.81	449.50	242.50	442.35	212.45	396.71
7.....	317.42	542.37	304.43	530.23	269.72	471.05
8.....	422.52	665.50	401.43	657.35	352.32	578.76
9.....	521.45	815.37	498.51	808.97	437.83	695.00
10.....	599.84	973.37	579.77	981.03	505.98	817.50
11.....	713.39	1,154.75	695.86	1,128.09	594.81	942.10
12.....	879.84	1,321.12	813.14	1,278.09	725.47	1,049.34
13.....	1,052.90	1,474.32	979.14	1,456.47	844.53	1,134.87
14.....	1,234.84	1,628.50	1,104.14	1,607.09	966.70	1,261.71
15.....	1,393.39	1,744.12	1,266.00	1,714.85	1,075.94	1,340.53
16.....	1,497.58	1,834.37	1,386.57	1,876.32	1,176.98	1,427.89
17.....	1,630.80	1,961.81	1,532.07	2,087.79	1,276.32	1,586.21
18.....	1,764.03	2,089.25	1,677.57	2,199.26	1,375.66	1,644.74
20.....	2,113.55	2,300.12	2,034.28	2,367.94	1,591.89	1,747.24
22.....	2,274.19	2,472.25	2,313.28	2,595.59	1,692.45	1,919.34
24.....	2,559.84	2,650.00	2,539.43	2,782.79	1,880.00	2,074.60
26.....	2,706.61	2,799.62	2,690.28	2,828.38	2,009.81	2,137.37
28.....	2,931.93	2,920.50	2,893.43	2,955.44	-----	-----
30.....	3,030.91	3,081.37	2,946.43	3,118.32	-----	-----
32.....	3,254.03	3,188.75	3,072.28	3,257.65	-----	-----
34.....	3,322.58	3,437.00	3,171.00	3,346.47	-----	-----
36.....	-----	-----	3,320.71	3,403.97	-----	-----
38.....	-----	-----	3,457.14	3,556.03	-----	-----

\* Caponized when 10 weeks old.

TABLE 2.—Statistical constants of the data given in Table 1

Age (weeks)	Statistical constants	Cockerels		Capons		Pullets	
		Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
0	{ P. E. of mean.gm... }	± 0.46	± 0.33	± 0.48	± 0.51	± 0.33	± 0.44
	{ σ.....gm... }	3.83± .33	3.15± .24	4.23± .34	4.41± .36	3.52± .23	4.04± .31
	{ C. of V...per cent... }	9.48± .81	7.91± .60	10.35± .84	10.83± .90	8.87± .58	10.30± .81
1	{ P. E. of mean.gm... }	± 1.18	± 1.19	± .87	± 1.26	± .87	± 1.16
	{ σ.....gm... }	9.58± .83	11.17± .84	7.56± .62	10.93± .89	9.43± .62	10.64± .82
	{ C. of V...per cent... }	14.98± 1.33	17.73± 1.38	12.02± 1.00	16.60± 1.40	15.24± 1.02	16.98± 1.35
2	{ P. E. of mean.gm... }	± 2.30	± 2.14	± 1.13	± 2.08	± 1.25	± 1.99
	{ σ.....gm... }	19.01± 1.63	20.05± 1.61	9.96± .80	18.01± 1.47	13.51± .88	18.15± 1.40
	{ C. of V...per cent... }	22.16± 1.99	20.21± 1.59	11.94± .98	18.33± 1.55	16.60± 1.12	19.31± 1.55
3	{ P. E. of mean.gm... }	± 3.15	± 3.64	± 2.12	± 3.65	± 1.80	± 3.55
	{ σ.....gm... }	25.99± 2.23	34.13± 2.57	18.32± 1.50	31.54± 2.58	19.09± 1.27	32.45± 2.51
	{ C. of V...per cent... }	23.57± 2.13	20.90± 1.64	17.19± 1.45	19.40± 1.65	18.35± 1.27	21.84± 1.77
4	{ P. E. of mean.gm... }	± 3.92	± 4.59	± 2.82	± 5.24	± 2.16	± 5.01
	{ σ.....gm... }	32.39± 2.77	43.01± 3.24	24.78± 2.00	45.34± 3.71	23.33± 1.53	45.81± 3.54
	{ C. of V...per cent... }	24.57± 2.23	19.70± 1.54	18.73± 1.56	20.63± 1.76	19.14± 1.30	23.61± 1.93

TABLE 2.—Statistical constants of the data given in Table 1—Continued

Age (weeks)	Statistical constants	Cockerels		Capons		Pullets	
		Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
5	{ P. E. of mean gm...	± 5.45	± 6.24	± 3.24	± 6.98	± 3.01	± 6.00
	{ σ.....per cent...	44.97± 3.85	58.49± 4.41	28.44± 2.29	60.31± 4.93	32.46± 2.13	54.86± 4.24
	{ C. of V...per cent...	25.85± 2.36	18.41± 1.44	16.37± 1.36	19.28± 1.64	20.54± 1.40	19.48± 1.56
6	{ P. E. of mean gm...	± 9.41	± 8.60	± 5.40	± 8.97	± 4.75	± 8.33
	{ σ.....per cent...	77.69± 6.65	80.67± 6.08	46.67± 3.82	77.57± 6.34	51.27± 3.36	76.16± 5.89
	{ C. of V...per cent...	31.60± 2.97	17.95± 1.40	19.24± 1.63	17.53± 1.48	24.13± 1.67	19.20± 1.54
7	{ P. E. of mean gm...	±13.01	± 8.93	± 6.67	± 8.21	± 5.99	± 7.95
	{ σ.....per cent...	107.40± 9.20	83.75± 6.31	58.50± 4.72	70.99± 5.81	64.66± 4.24	72.70± 5.62
	{ C. of V...per cent...	33.83± 3.21	15.44± 1.19	19.22± 1.62	13.39± 1.12	23.97± 1.66	15.43± 1.22
8	{ P. E. of mean gm...	±15.70	±10.35	± 8.69	± 9.27	± 8.58	± 9.43
	{ σ.....per cent...	129.58±11.10	97.06± 7.32	76.21± 6.14	80.10± 6.55	91.79± 6.01	86.21± 6.67
	{ C. of V...per cent...	30.67± 2.86	14.58± 1.12	18.98± 1.58	12.18± 1.01	26.05± 1.82	14.89± 1.18
9	{ P. E. of mean gm...	±17.81	±12.35	±10.38	±10.12	±10.21	±10.43
	{ σ.....per cent...	147.03±12.59	115.77± 8.73	91.04± 7.34	87.48± 7.15	110.22± 7.22	95.37± 7.38
	{ C. of V...per cent...	28.20± 2.60	14.20± 1.09	18.26± 1.52	10.81± .90	25.17± 1.75	13.72± 1.08
10	{ P. E. of mean gm...	±19.45	±13.62	±11.11	±12.66	±11.27	±12.42
	{ σ.....per cent...	160.56±13.75	127.72± 9.63	97.48± 7.86	109.43± 8.95	121.69± 7.97	113.47± 8.78
	{ C. of V...per cent...	26.76± 2.45	13.12± 1.01	16.81± 1.39	11.15± .92	24.05± 1.61	13.88± 1.09
11	{ P. E. of mean gm...	±21.39	±15.85	±12.98	±14.50	±12.84	±13.77
	{ σ.....per cent...	176.56±15.12	148.58±11.20	113.82± 9.18	125.33±10.25	138.58± 9.08	125.83± 9.74
	{ C. of V...per cent...	20.75± 2.25	12.87± .99	16.36± 1.35	11.11± .92	23.30± 1.61	13.36± 1.05
12	{ P. E. of mean gm...	±27.73	±19.18	±15.94	±16.11	±14.83	±14.57
	{ σ.....per cent...	228.91±19.61	179.84±13.56	139.80±11.27	139.29±11.39	160.10±10.49	133.12±10.30
	{ C. of V...per cent...	25.02± 2.38	13.61± 1.05	17.19± 1.43	10.90± .86	22.07± 1.52	12.69± 1.00
13	{ P. E. of mean gm...	±29.87	±16.85	±17.84	±17.52	±15.83	±17.16
	{ σ.....per cent...	246.57±21.12	157.96±11.91	156.46±12.61	151.43±12.39	170.86±11.19	156.84±12.13
	{ C. of V...per cent...	23.42± 2.37	10.71± .82	15.98± 1.32	10.40± .86	20.23± 1.38	13.82± 1.09
14	{ P. E. of mean gm...	±32.30	±19.62	±18.55	±19.75	±16.19	±18.61
	{ σ.....per cent...	296.58±22.83	183.96±13.87	162.69±13.12	170.73±13.96	174.71±11.45	170.12±13.16
	{ C. of V...per cent...	21.59± 1.93	11.30± .86	14.73± 1.21	10.62± .88	18.07± 1.22	13.48± 1.06
18	{ P. E. of mean gm...	±38.92	±23.66	±23.88	±27.36	±17.16	±21.38
	{ σ.....per cent...	321.27±27.52	221.85±16.73	209.50±16.89	236.53±19.35	185.20±12.13	222.80±17.24
	{ C. of V...per cent...	18.21± 1.61	10.62± .81	12.49± 1.02	10.75± .89	13.46± .90	13.55± 1.07
22	{ P. E. of mean gm...	±37.70	±25.42	±30.16	±33.97	±18.46	±24.82
	{ σ.....per cent...	311.14±32.65	238.30±17.97	264.50±21.32	293.63±24.02	199.24±13.05	263.40±20.38
	{ C. of V...per cent...	13.68± 1.19	9.64± .73	11.43± .93	11.31± .94	11.77± .78	13.72± 1.08
26	{ P. E. of mean gm...	±43.81	±27.20	±35.76	±39.77	±22.29	±32.35
	{ σ.....per cent...	361.63±30.98	255.00±19.23	313.69±25.29	343.73±24.12	240.61±15.76	295.60±22.87
	{ C. of V...per cent...	13.36± 1.17	9.11± .69	11.79± .96	12.15± 1.01	11.97± .80	13.83± 1.09
30	{ P. E. of mean gm...	±48.49	±31.80	±41.44	±41.24	-----	-----
	{ σ.....per cent...	400.24±34.28	298.17±22.49	363.45±29.30	356.49±20.16	-----	-----
	{ C. of V...per cent...	13.12± 1.14	9.68± .73	12.34± 1.01	11.43± .95	-----	-----
34	{ P. E. of mean gm...	±46.53	±31.41	±48.88	±42.46	-----	-----
	{ σ.....per cent...	384.01±32.90	294.46±22.21	428.68±34.56	367.05±30.02	-----	-----
	{ C. of V...per cent...	11.56± 1.00	8.57± .65	13.52± 1.11	11.00± .91	-----	-----
38	{ P. E. of mean gm...	±-----	±-----	±55.53	±49.66	-----	-----
	{ σ.....per cent...	-----	-----	487.07±39.27	429.26±35.11	-----	-----
	{ C. of V...per cent...	-----	-----	14.09± 1.16	12.07± 1.00	-----	-----

After these data had been assembled it seemed desirable to compare the growth of the several lots of Rhode Island Red chicks with the growth of chicks of the same breed as published by other investigators. A search of the literature revealed the fact that there were but few instances in which complete, or even approximately complete, growth data had been obtained and published for this breed.

In 1918 Card and Kirkpatrick (11)<sup>2</sup> reported growth data for four lots of Rhode Island Red chicks from which the cockerels were removed on the twelfth, sixteenth, thirteenth, and twelfth weeks, respectively. Three years later Brody (4) used the average growth data of these four lots in a study of the rate of growth of the domestic fowl. Robertson (28) in turn, a few years later, cited Brody's analysis in support of his contention that the growth of animals may be

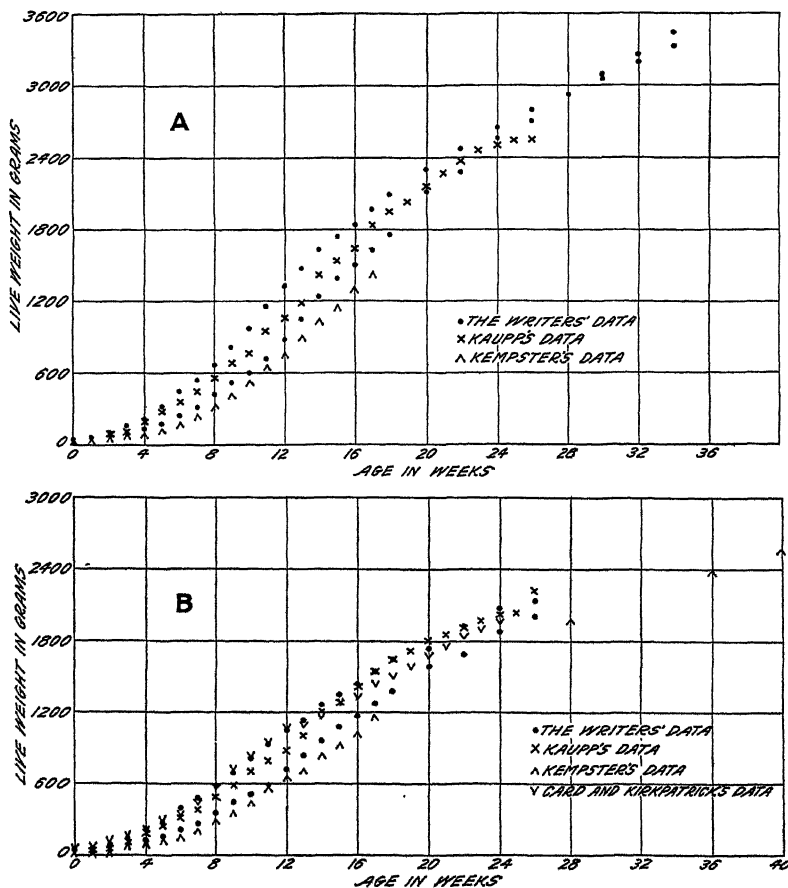


FIG. 1.—Comparison of the writers' growth data for Rhode Island Red chickens with the growth data obtained by other investigators for the same breed. A, cockerels; B, pullets. In each case the upper series of dots (.) represents the growth of the lot which received skim milk while the lower series of dots is for the lot which did not.

represented by the formula for a monomolecular, autocatalytic, chemical reaction. In the same year (1923) in which Robertson's monograph appeared Kempster and Henderson (16) compared these data obtained by Card and Kirkpatrick with some published by Kaupp (15) in 1921.

Kaupp reported in 1921 the average growth of several lots of Rhode Island Red chicks by sexes. In obtaining his data he fed

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 539.

similar rations to the several lots, the difference between the rations being that they contained different protein supplements, i. e., milk, meat scraps, and tankage.

In 1926 Kempster and Seaton (17) reported the growth, by sexes, of Rhode Island Red chicks up to the age of 17 weeks.

The writers plotted the several sets of data mentioned above with the data in Figure 1, A and B. In the case of the cockerels the average growth of Kaupp's several lots falls between the growth of the writers' lot receiving skim milk and the one not receiving it. The growth of Kempster's chicks is appreciably less than that of the writers' chicks which received no milk.

In the case of the pullets the average growth of Kaupp's chicks falls between that of the writers' two lots until the sixteenth week, after which it is similar to that of the writers' lot receiving milk. Again the growth of Kempster's chicks is less than that of those receiving no milk in the present investigation. For the first 12 weeks the growth of Card and Kirkpatrick's chicks (males and females) was very similar to the growth of the writers' pullets, but after that time the growth of their chicks (females) fell between that of the writers' two lots.

In general, the growth of cockerels and pullets in the studies here reported appears to be more nearly uniform than the growth of the cockerels and pullets of the other investigators; also, for the ages plotted, the writers' cockerels receiving skim milk were heavier than any of the others, and the pullets receiving skim milk were as heavy as the heaviest of either Kaupp's or Card and Kirkpatrick's pullets.

Inasmuch as a comparison of the writers' data with the data secured by other investigators indicates the great difficulty, if not impossibility, of setting up a "normal growth curve" for a given breed of chickens, the data given here are submitted, not as representing the "normal growth" of the Rhode Island Red breed but rather as representing the growth which was observed by the writers for their strain of that breed.

To show how the variability of the live weight of the chicks changed with increasing age, the coefficient of variation of the live weight of the chicks of each lot was plotted against age in Figure 2. (See Table 2.) In each case the resulting curve shows a characteristic hook shape (C—). For the three lots receiving skim milk (lot 2 cockerels, lot 2 capons, and lot 2 pullets) the period of high variability in live weight is confined to the first seven or eight weeks, whereas for the three lots not receiving skim milk (lot 1 cockerels, lot 1 capons, and lot 1 pullets) the period of high variability extends to at least the twelfth week.

Jull (14) has called attention to this increase in variability in live weight during the first four or five weeks in the case of Barred Plymouth Rock chicks. He found that after this period of high variability, which lasted until the tenth week for males and twelfth week for females, there was a general tendency toward decrease in variability in live weight for both sexes. In the case of each of the writers' lots of chicks the variability also showed a general tendency to decrease with age, and after the twenty-second week lay well between 7.5 and 15 per cent.

In regard to the relationship between sex and variability in live weight, the writers' data show that for the three lots (cockerels,

capons, and pullets) receiving skim milk the period of high variability, as well as the extent of the variation during this period, is very much the same; however, in the case of the three lots receiving no skim milk the period of high variability is about the same, but the extent of the variation during this period is much greater for the cockerels than the pullets and much greater for the pullets than the capons. No significance is attached to these differences in extent of variability because, until the age of 10 weeks, the chicks designated as capons were cockerels and it was before this age that the difference between lot 1 cockerels and lot 1 capons became most pronounced.

In many nutrition experiments with poultry growth is the main criterion for judging and analyzing the results. When the Rhode Island Red is used the writers' determinations of the probable error of the mean live weight for the different ages should give some indication of the probable error of the mean live weight to be expected

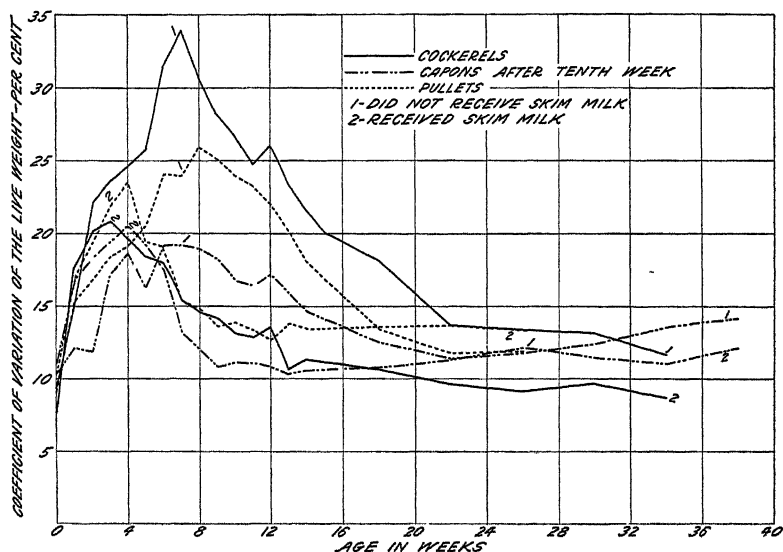


FIG. 2.—The variability of live weight with age

for normal lots of from 30 to 50 chicks. There is but one other breed, the Barred Plymouth Rock, known to the writers, for which the probable error of the mean live weight at different ages may be found in the literature; Jull (14) gives the probable error of the mean live weight of lots containing 38 of each sex of this breed for the first 22 weeks.

In a recent contribution to the study of growth Brody (6) calls attention to the sharp breaks frequently observed when time rates of growth are plotted and suggests that these are breaks between successive stages of constant growth rates, i. e., between successive cycles of growth. In the course of analyzing the present data the writers plotted the standard deviation of the weights of the chicks in each lot against time (age) and found analogous breaks. In many instances the plotted points fell approximately along a straight line for a period of several weeks and then there was a break and again

the plotted points fell approximately along a straight line; in the case of each lot of chicks there were four or five periods in which the plotted points fell approximately along straight lines. The standard deviation, when plotted against age, may give valuable clues as to the approximate time of the beginning and end of cycles of growth.

#### THE GROWTH CURVES

Many more or less successful attempts have been made to describe the growth of animals, as well as of plants, by means of mathematical formulas. Some investigators have been content to use wholly empirical formulas while others have attempted to derive rational ones. Robertson (28) has called attention to the similarity in shape of the curve of the monomolecular, autocatalyzed, chemical reaction and the curve of normal growth of animals and has attempted to describe the growth of animals (and plants) by means of the equation of this curve. Brody (5, 7), observing that the successive periodic gains in the live weight of an animal seemed to bear a definite relationship to each other during the later stages of growth, employed the curve of diminishing increment in analyzing the growth of several species of animals.

It is not the present purpose to review the large amount of work which has been done in this field or to discuss the relative value of the several formulas which have been proposed by different investigators. Persons interested in this phase of the subject are referred to the numerous papers of Robertson and of Brody.

In the present study the writers selected the curves used by the above-mentioned investigators because those curves had been used in more instances than any others for describing the growth of chickens. The autocatalytic formula was selected for describing the average growth of the lots of chicks during the first 15 to 18 weeks and the formula of the curve of diminishing increment for the remainder of the growth.

In the course of the curve fitting the writers found that the autocatalytic curve did not describe the growth of the lots of chicks so well as had been hoped. So far as the writers' data are concerned, the autocatalytic formula is only an approximation for a part of the growth curve. This is in agreement with the contention of Van de Sande-Bakhuyzen (29, 30) that the autocatalytic formula is only a first approximation for a part of the growth curve. It may also be remarked that Murray (22) has raised a number of objections to the autocatalytic concept of growth. However, despite the objections that have been raised against the autocatalytic formula for describing growth and the fact that the autocatalytic curve did not describe the average growth of the lots of chicks so well as had been hoped, the writers feel that the constants which were obtained in fitting this curve to the sets of data are of significance, at least empirically.

The curve of diminishing increment fully met the writers' expectations as to its suitability for describing the later stages of the growth of the six lots of chickens.

Before reporting the results of the curve fitting, a short description of the method used may be of interest. First, the constants for each curve, for each lot of chicks, were approximately determined by a combination of graphical and least square methods described by

Robertson (28) and by Brody (5). In the case of the autocatalytic curve  $\left(\log \frac{w}{A-w} = K(t-t_1),\right.$

in which

$w$  = weight of the animal at any time  $t$ ,  
 $A$  = weight which the animal approaches asymptotically as the cycle of growth involved approaches completion.  
 $K$  = the velocity constant of growth,  
 $t$  = age, and  
 $t_1$  = age at which the cycle is half completed, i. e., when  $w = \frac{A}{2}$ ,

the accuracy of the determination of the constants  $K$  and  $t_1$  depends on the accuracy with which the constant  $A$  has been determined graphically; and in the case of the curve of diminishing increment<sup>3</sup>

$$(w = A - B\epsilon^{-k(t+3)},$$

in which

$w$  = weight of the animal at any time  $t$ ,  
 $A$  = mature weight of animal which is approached asymptotically as  $t$  approaches infinity,  
 $B$  = a constant upon which the gain in weight of the animal is dependent and which in turn (as we believe) is dependent upon the feed which the animal receives as well as the kind of animal,  
 $\epsilon$  = the base of the natural system of logarithms,  
 $k$  = a constant indicating the rate of decline in growth of the animal,  
 $t$  = age from time of hatching (birth),  
 $3$  = number of weeks required for hatching (period of gestation).)

the accuracy of the determination of the constants  $B$  and  $k$  depends on the accuracy with which the constant  $A$  has been determined graphically.

Desiring extreme accuracy in the determination of the constants, the writers corrected them in the manner described by Robertson (28) in the appendix of his monograph on the chemical basis of growth and senescence. Finding that in most cases these corrections ranged from a fraction of a per cent to as much as 10 per cent, the writers continued to correct the "corrected" constants until negligible corrections were obtained.

Inasmuch as the writers' "correction" or adjustment equation was not the same as the one in Robertson's monograph, there is given here

<sup>3</sup> The curve of diminishing increment is sometimes written in the form,  $w = A - BR^t$ , or in the present case:

$w = A - BR^{(t+3)},$

in which  $w$ ,  $A$ ,  $B$ ,  $t$ , and  $3$  have the same significance as in the other form and  $R = \epsilon^{-k}$ . In this form of the equation the increase in weight during any period of time (week, if  $t$  is measured in weeks, etc.) is  $R$  (always a fraction) times that of the preceding period.

the adjustment equation used with each type of curve. For the autocatalytic equation the writers used:<sup>4</sup>

$$\frac{d\phi}{dA} \alpha + \frac{d\phi}{dK} \beta + \frac{d\phi}{dt_1} \gamma = \theta$$

in which

$$\frac{d\rho}{dA} = \frac{x}{A},$$

$$\frac{d\phi}{dK} = (t - t_1) A V,$$

$$\frac{d\phi}{dt_1} = -KA V, \text{ and}$$

$$V = \frac{2.302585 \cdot 10^{K(t-t_1)}}{[1 + 10^{K(t-t_1)^2}]}$$

In Robertson's adjustment equation<sup>5</sup>  $\frac{d\phi}{dK} = t A V$ , while in the writers'  $\frac{d\rho}{dK} = (t - t_1) A V$ .

For the curve of diminishing increment the writers used:<sup>6</sup>

$$\frac{d\phi}{dA} \alpha + \frac{d\phi}{dB} \beta + \frac{d\phi}{dK} \gamma = \theta$$

in which:

$$\frac{d\phi}{dA} = 1,$$

$$\frac{d\phi}{dB} = -\epsilon^{-k(t+3)} \text{ or } -\frac{1}{\epsilon^{k(t+3)}}, \text{ and}$$

$$\frac{d\phi}{dK} = (t+3) B \epsilon^{-k(t+3)}$$

The constants for each curve, for each lot of chicks, as finally determined are given in Tables 5 and 6.

TABLE 3.—*Constants of the autocatalytic curve<sup>a</sup> fitted to the first 15 to 18 weeks of growth*

Constants	Cockerels		Capons		Pullets	
	Lot 1 (0-18 weeks)	Lot 2 (0-16 weeks)	Lot 1 (0-18 weeks)	Lot 2 (0-15 weeks)	Lot 1 (0-15 weeks)	Lot 2 (0-16 weeks)
A (grams).....	2, 234	2, 133	2, 250	2, 090	1, 747	1, 599
K.....	0.1238	0.1414	0.1141	0.1412	0.1179	0.1390
t <sub>1</sub> (weeks).....	13.40	10.46	14.06	10.43	13.27	9.87
S <sup>b</sup> (grams).....	±16.66	±18.70	±13.25	±19.16	±8.79	±19.27

<sup>a</sup>  $\log \frac{w}{A-w} = K(t-t_1)$

<sup>b</sup> S=root-mean-square deviation of the observed weights from the calculated weights.

<sup>4</sup> Employing Robertson's system of notation (§8).

<sup>5</sup> Probably due to a typographical error.

<sup>6</sup> Employing Robertson's system of notation (§8).



TABLE 4.—Constants of the curve of diminishing increment <sup>a</sup> fitted to the later stages of growth

Constants	Cockerels		Capons		Pullets	
	Lot 1 (16-32 weeks)	Lot 2 (14-32 weeks)	Lot 1 (14-38 weeks)	Lot 2 (13-38 weeks)	Lot 1 (12-26 weeks)	Lot 2 (11-26 weeks)
A (grams).....	4, 480	4, 438	4, 035	4, 063	3, 385	3, 316
B (grams).....	8, 308	6, 086	8, 992	6, 950	5, 388	4, 682
k.....	0. 0538	0. 0452	0. 0650	0. 0612	0. 0471	0. 0482
t <sup>b</sup> (weeks).....	8. 48	3. 98	9. 32	5. 76	6. 87	4. 15
R <sup>c</sup> .....	0. 9477	0. 9551	0. 9370	0. 9406	0. 9540	0. 9529
S <sup>d</sup> (grams).....	±29. 74	±12. 08	±42. 78	±36. 51	±14. 13	±17. 95

<sup>a</sup>  $w = A - Be^{-k(t+t^*)}$  or  $w = A - BR(t+t^*)$   
<sup>b</sup>  $t^*$  = age at which  $w=0$ . See Brody (5, 7).  
<sup>c</sup>  $R = e^{-k}$ , i. e.,  $R$  is the ratio of the gain in live weight during any week to that during the preceding week.  
<sup>d</sup>  $S$  = root-mean-square-deviation of the observed weights from the calculated weights.

Having determined the constants of the two curves for each lot of chicks, the writers calculated the weights for each week and plotted the curves together with the observed weights. (Figs. 3, A; 4, A; 5, A; 6, A; 7, A; 8, A.) The calculated weights are given in Tables 5 and 6.

TABLE 5.—Weights of the chicks for the first 15 to 18 weeks of growth, calculated by means of the formula for the autocatalytic curve

Age (weeks)	Weight of—					
	Cockerels		Capons		Pullets	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
	Grams	Grams	Grams	Grams	Grams	Grams
0.....	48	68	55	68	46	65
1.....	63	94	70	93	60	88
2.....	83	128	91	127	78	119
3.....	110	173	117	171	101	160
4.....	143	232	149	230	131	212
5.....	187	308	190	306	168	278
6.....	242	405	242	401	213	359
7.....	310	522	304	517	270	456
8.....	394	661	380	653	338	567
9.....	496	818	471	807	418	689
10.....	614	987	576	973	510	816
11.....	749	1, 160	695	1, 143	613	943
12.....	897	1, 329	827	1, 308	725	1, 062
13.....	1, 053	1, 484	969	1, 459	842	1, 170
14.....	1, 212	1, 621	1, 115	1, 593	960	1, 263
15.....	1, 367	1, 737	1, 262	1, 706	1, 075	1, 340
16.....	1, 512	1, 832	1, 405	-----	-----	1, 402
17.....	1, 644	-----	1, 538	-----	-----	-----
18.....	1, 759	-----	1, 660	-----	-----	-----

In order to visualize better the average growth of the six lots of chicks, the writers plotted the observed average weekly gains against age, as well as the smoothed <sup>7</sup> average weekly gains and the calculated average weekly gains.<sup>8</sup> (Figs. 3, B; 4, B; 5, B; 6, B; 7, B; and 8, B.) In this way some interesting points were brought out which will be discussed later.

<sup>7</sup> Method of moving averages.

<sup>8</sup> Not the instantaneous rates of gain.

TABLE 6.—Weights of the chicks for the later stages of growth, calculated by means of the formula for the curve of diminishing increment <sup>a</sup>

Age (weeks)	Weight of—					
	Cockereels		Capons		Pullets	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
	Grams	Grams	Grams	Grams	Grams	Grams
11.....					(597)	933
12.....					725	1,045
13.....			(858)	1,454	847	1,152
14.....	(1,149)	1,618	1,058	1,609	964	1,254
15.....	(1,323)	1,743	1,246	1,755	1,075	1,351
16.....	1,489	1,862	1,421	1,892	1,182	1,444
17.....	1,645	1,976	1,586	2,021	1,283	1,532
18.....	1,793	2,085	1,740	2,143	1,379	1,616
19.....	1,934	2,189	1,884	2,257	1,472	1,696
20.....	2,067	2,288	2,020	2,364	1,560	1,772
21.....	2,194	2,383	2,147	2,465	1,644	1,845
22.....	2,313	2,474	2,266	2,560	1,724	1,914
23.....	2,427	2,561	2,377	2,649	1,800	1,980
24.....	2,534	2,644	2,481	2,733	1,873	2,043
25.....	2,636	2,723	2,579	2,812	1,942	2,103
26.....	2,733	2,799	2,671	2,886	2,009	2,160
27.....	2,824	2,872	2,757	2,956	(2,072)	(2,214)
28.....	2,911	2,941	2,837	3,022	(2,132)	(2,266)
29.....	2,993	3,007	2,913	3,084	(2,190)	(2,316)
30.....	3,071	3,071	2,983	3,142	(2,245)	(2,363)
31.....	3,144	3,131	3,049	3,197	(2,297)	(2,408)
32.....	3,214	3,189	3,112	3,248	(2,347)	(2,451)
33.....	3,281	(3,244)	3,170	3,297	(2,395)	(2,491)
34.....	3,343	(3,297)	3,224	3,342	(2,440)	(2,530)
35.....	(3,403)	(3,347)	3,275	3,385		
36.....	(3,459)	(3,396)	3,323	3,425	(2,525)	(2,602)
37.....	(3,513)	(3,442)	3,368	3,463		
38.....	(3,563)	(3,486)	3,410	3,499	(2,603)	(2,668)
39.....	(3,611)	(3,528)	(3,449)	(3,532)		
40.....	(3,657)	(3,568)	(3,480)	(3,564)	(2,673)	(2,728)

<sup>a</sup> The weights in parentheses are extrapolated values; i. e., they lie outside the age intervals to which the curves were fitted.

A casual inspection of Figures 3 to 8, inclusive, leaves an impression that, in general, the curves selected to describe the growth of the chicks fulfilled this purpose rather well. As a comparison of the observed with the calculated weights (Tables 1 and 6) shows, this is true in the case of the curve of diminishing increment; however, in the case of the autocatalytic curve the agreement between the observed and calculated weights is not so good for the first several weeks, except, possibly, in the case of lot 1 cockereels and lot 1 pullets. (Compare Tables 1 and 5.)

This is contrary to the results obtained by Brody (4) in fitting this curve to Card and Kirkpatrick's (11) growth data for the Rhode Island Red chicken, for in this instance the agreement between the observed and calculated weights was unusually close. Since environmental, as well as nutritional, conditions are such potent factors in the early growth of chickens, it seems almost too much to expect that any one type of curve would be able to describe their early growth unless these conditions were rigidly controlled. It is therefore to be anticipated that there will be cases in which a given type of curve will accurately describe the first several weeks of the growth of chickens as well as cases in which it will fail to do so.

A study of Tables 3 and 4 shows that in each case the constants of the growth curves were markedly affected by allowing the birds

free access to sour skim milk in addition to feeding them the basal ration. In the case of the constants of the autocatalytic curve, the constant  $A^0$  was depressed 4.5 per cent (cockerels) to 8.5 per cent

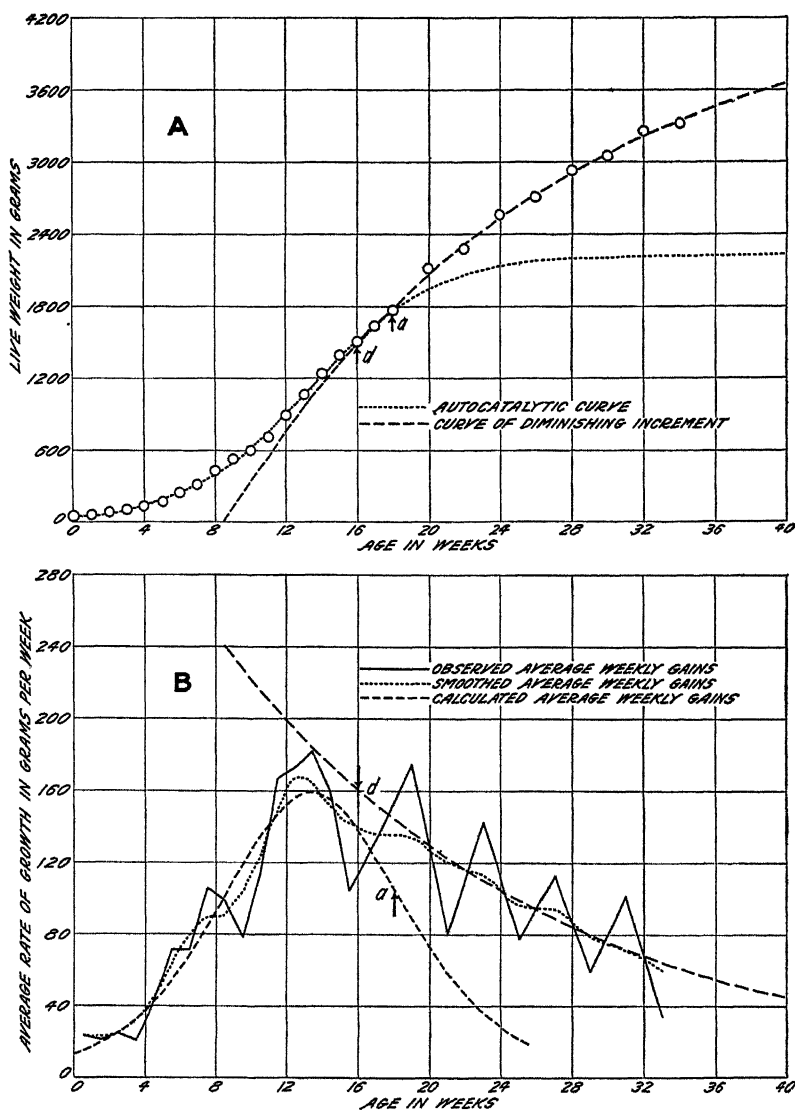


FIG. 3.—Lot 1 cockerels. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case  $a$  marks the end of the age interval to which the autocatalytic curve was fitted and  $d$  marks the beginning of the age interval to which the curve of diminishing increment was fitted.

(pullets), but the velocity constant of growth  $K$  was increased 13.4 per cent (cockerels) to 23.76 per cent (capons), and the time  $t_1$  required to make one-half of the growth indicated by constant  $A$

<sup>9</sup> For the explanation of the constants discussed, see p. 523.

was decreased 21.9 per cent (cockerels) to 25.8 per cent (capons). That is, the feeding of sour skim milk slightly decreased the amount of growth indicated by the constant  $A$  but very greatly decreased the time required to make that growth. In brief, then, it may be stated that feeding sour skim milk greatly accelerated growth during the first 15 to 18 weeks.

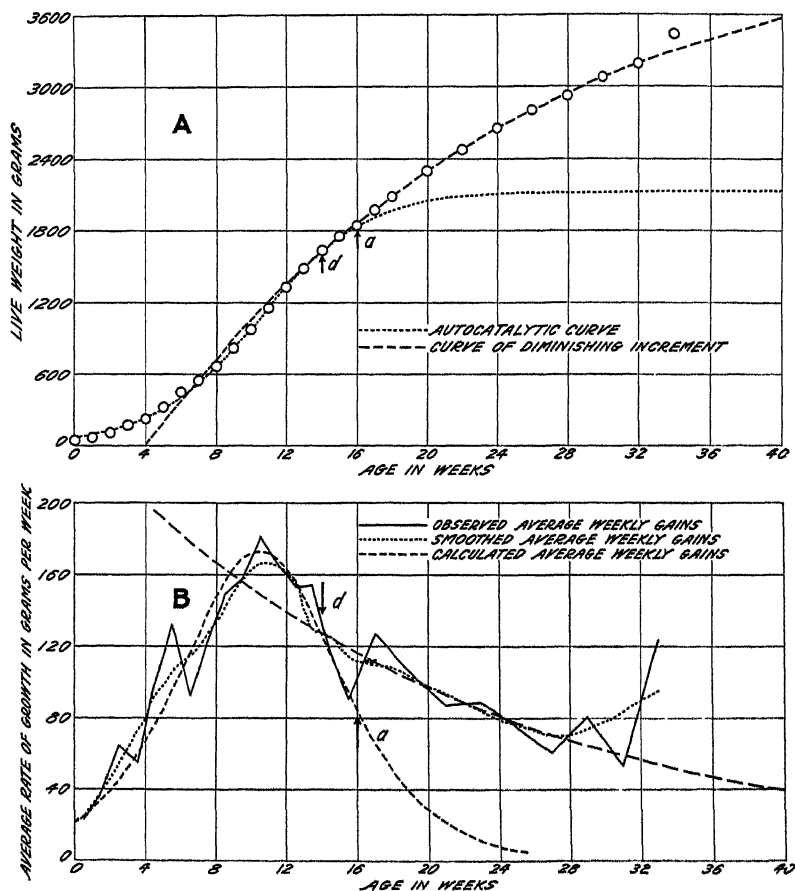


FIG. 4.—Lot 2 cockerels. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case  $a$  marks the end of the age interval to which the autocatalytic curve was fitted and  $d$  marks the beginning of the interval to which the curve of diminishing increment was fitted

In the case of the curve of diminishing increment the average mature weight of the chickens, as represented by the constant  $A$ , was not greatly affected, but the constant  $B$ , upon which the magnitude of the absolute weekly gains is dependent, was very greatly decreased. As for  $k$ , considered by itself, it seems that the feeding of sour skim milk had no consistent effect on it. In each case  $t^*$  was greatly decreased in value.  $R$  appears to have remained rather constant not only for the different nutritional conditions but also for the different sexes; this is to be expected from the relationship between

$R$  and  $k$ , rather large changes in  $k$  corresponding to rather small changes (percentage basis) in  $R$ .

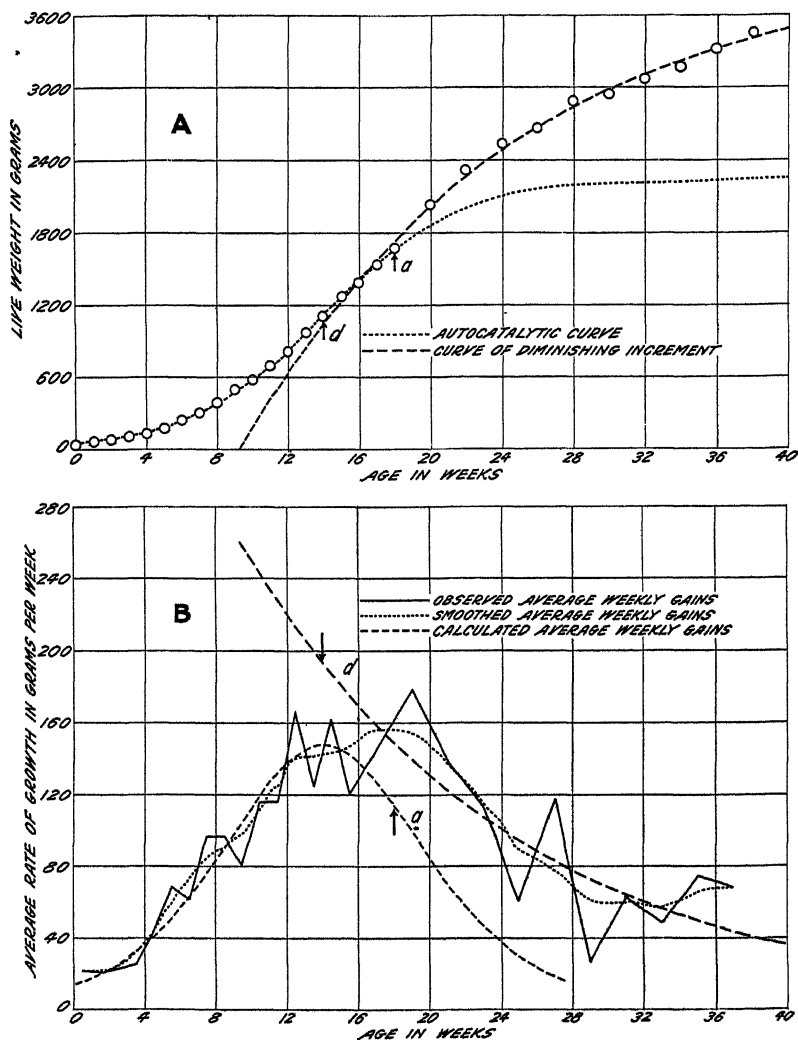


FIG. 5.—Lot 1 capons. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case  $a$  marks the end of the age interval to which the autocatalytic curve was fitted and  $d$  marks the beginning of the interval to which the curve of diminishing increment was fitted.

## GENERAL DISCUSSION

### THE EFFECT OF MILK ON GROWTH OF CHICKENS

Investigators for at least the last 35 years have loudly praised the value of milk (usually skim milk<sup>10</sup>) for feeding poultry. In 1898

<sup>10</sup> According to Card (10), buttermilk and sour skim milk have approximately the same feeding value. Except in extreme cases, it is probable that all the forms of milk usually fed to poultry have approximately the same feeding value.

Anderson (1) concluded that skim milk was especially valuable as a food for young chickens, but became of less importance as the chickens grew older. After repeating his experiments (2) he concluded that skim milk did not decrease in value as the chickens became older.

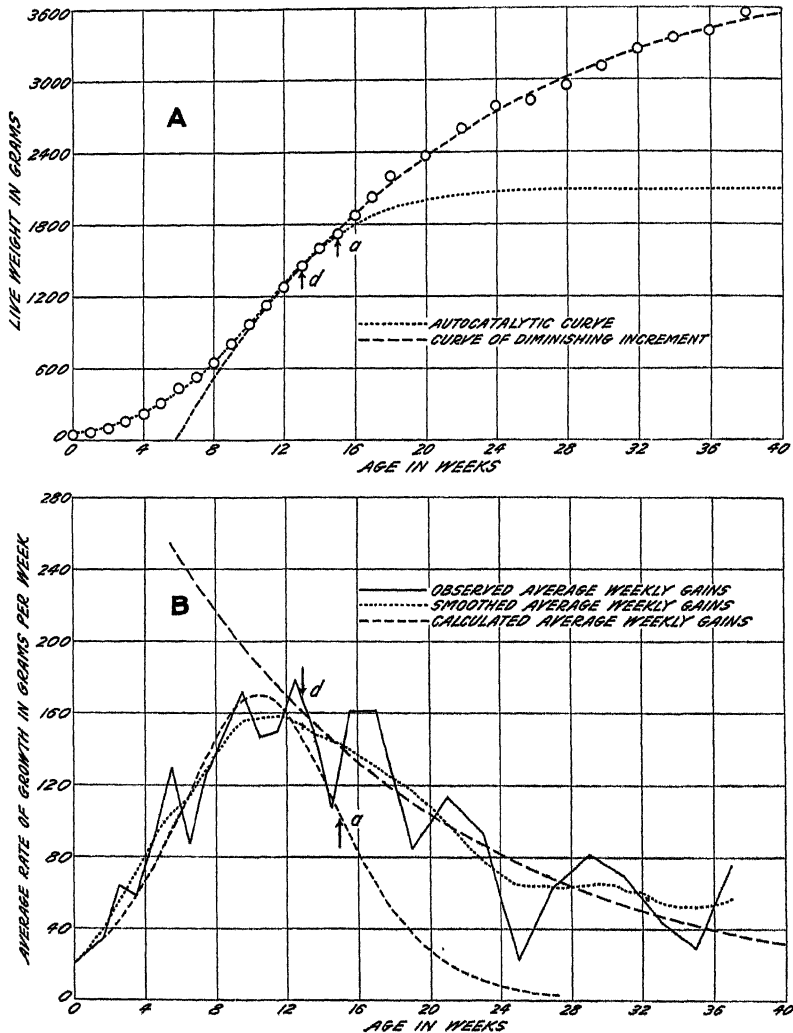


FIG. 6.—Lot 2 capons. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case *a* marks the end of the age interval to which the autocatalytic curve was fitted and *d* marks the beginning of the interval to which the curve of diminishing increment was fitted.

It seems to the writers that the value of skim milk, at any time, would depend to a great extent on the feed fed with it. The writers' results fit his first conclusion regarding age rather than his second. The basal ration which he fed the second year was not quite the same as that fed the first year and probably not so adequate for growth.

Wheeler (32) made a comparison of rations wholly of vegetable origin and others containing feed of animal origin, and from his results concluded that after the period of most active growth had passed and the young chickens were approaching maturity the difference in efficiency between such rations rapidly disappeared. His results parallel those of the writers.

Previous to Anderson's experiments, work done at the New York station (23) indicated the generally beneficial effect of skim milk on health and mortality. Rettger, Kirkpatrick, and Card (27) in 1915

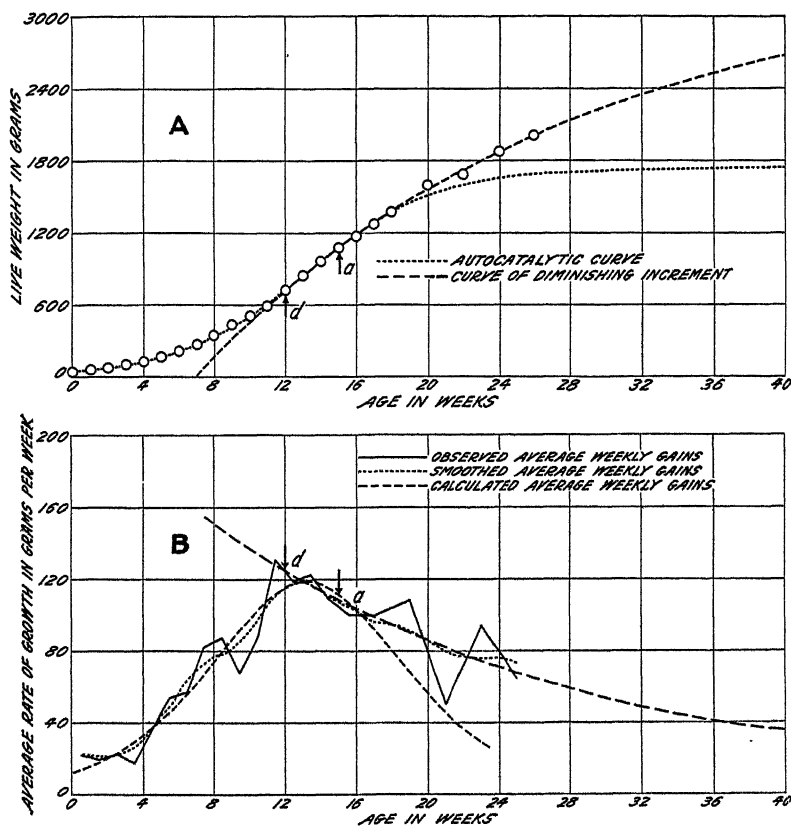


FIG. 7.—Lot 1 pullets. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case *a* marks the end of the age interval to which the autocatalytic curve was fitted and *d* marks the beginning of the interval to which the curve of diminishing increment was fitted

stated that it was most conclusively shown in all their experiments that milk feeding stimulated growth and caused a great reduction in deaths from general causes. Since then a number of investigators, Goodale (13), Lewis (19), Orr (24), and others, have testified to the beneficial effect of milk on growth.

Considering it as an established fact that milk does have a marked effect on the growth of chickens, it is desirable to know how, when, and why this effect is manifested. In Figures 9, A and B, 10, A and B, and 11, A and B, the writers have plotted the growth curves and

the smoothed average-rate-of-growth curves of the cockerels, capons, and pullets to show the effect of feeding milk. In each of the three cases the cumulative growth curves diverge from each other after the first week, but after the twenty-eighth week they either come together again or else show a marked tendency to do so. Likewise, in each of the three cases, the smoothed average-rate-of-growth curves separate at the start but in this instance they come together again and cross at about 12 weeks for the cockerels, 14.5 weeks for capons, and 11.5 weeks for pullets.

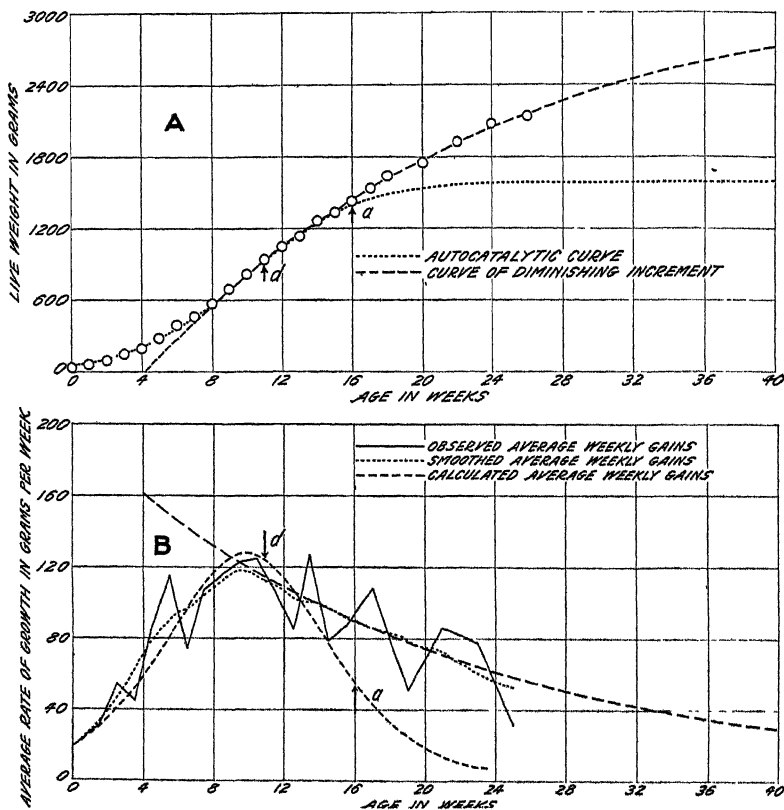


FIG. 8.—Lot 2 pullets. A, growth curves fitted to the observed average weights; B, average-rate-of-growth curves. In each case *a* marks the end of the age interval to which the autocatalytic curve was fitted and *d* marks the beginning of the interval to which the curve of diminishing increment was fitted

On examining the calculated average-rate-of-growth curves for the first 15 to 18 weeks (figs. 3 to 8, inclusive) one finds that in each case the curve for the lots receiving skim milk is higher and narrower than for the lots not receiving it. It is clear, then, according to the data, that the effect of skim milk on growth is shown by an increased rate of growth during the first 12 to 15 weeks.

Regarding the cause of this effect there is much speculation and uncertainty. In general, the cause is ascribed to the mineral and protein content of the milk, particularly the latter. Buckner,



Nollau, Wilkins, and Kastle (8) believed that the deficiency of certain grain rations for growth was due to the quality of the protein. In an earlier contribution of Buckner, Wilkins, and Kastle (9) some experiments were reported which indicated that the lysine content of certain grain mixtures was the limiting factor for growth. Although these experiments were open to criticism, Osborne and Mendel (25) later in part corroborated their findings. It may be that milk is

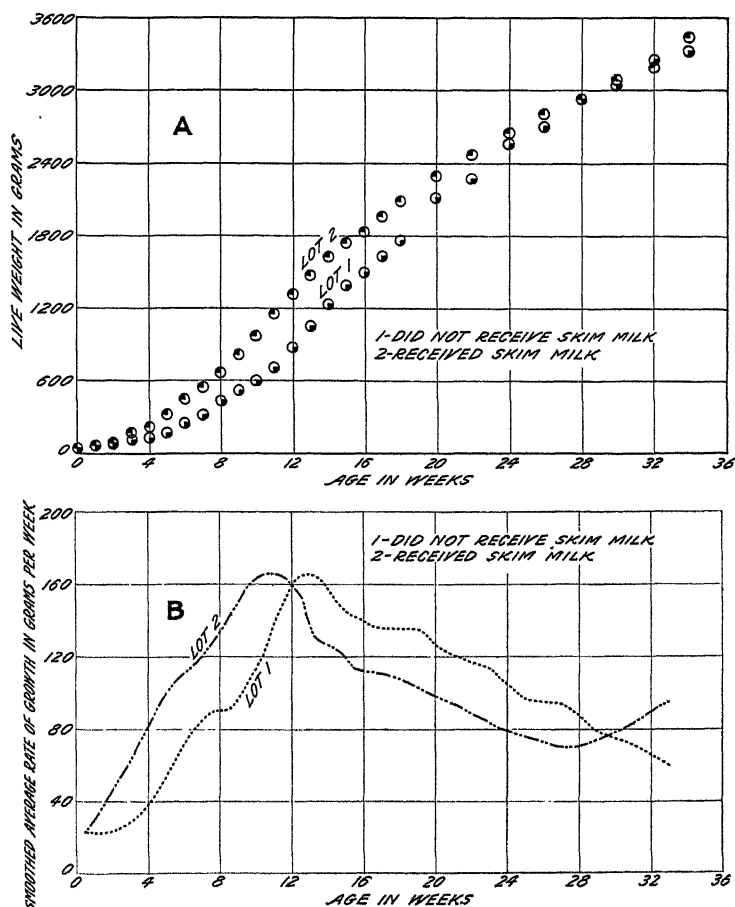


FIG. 9.—Comparison of the growth of the cockerels receiving skim milk with those not receiving it. A, cumulative growth curves; B, smoothed average-rate-of-growth curves

particularly valuable because of its proteins, and it is also possible that its value is due in part to its mineral content. One very striking thing is that feeding milk to chickens nearly always, if not always, increases the total feed consumption, and this in itself would tend to cause more rapid growth. However, it is easy to fall into the error of mistaking cause for effect, and if one assumes that milk is valuable because it increases feed consumption he is immediately called upon to explain why it does this.

Brody (7) has used the values of  $t^*$  (Table 4) for different animals (mammals) in comparing their equivalence of age. As the writers take it, he has assumed that  $t^*$  has a more or less fixed value for each kind of animal. According to the writers' values of  $t^*$ , this assumption as to constancy does not hold for Rhode Island Red chickens. However, if the hypothesis is applied to nutritionally different lots of chicks of the same breed, the writers' values of  $t^{*11}$  indicate that

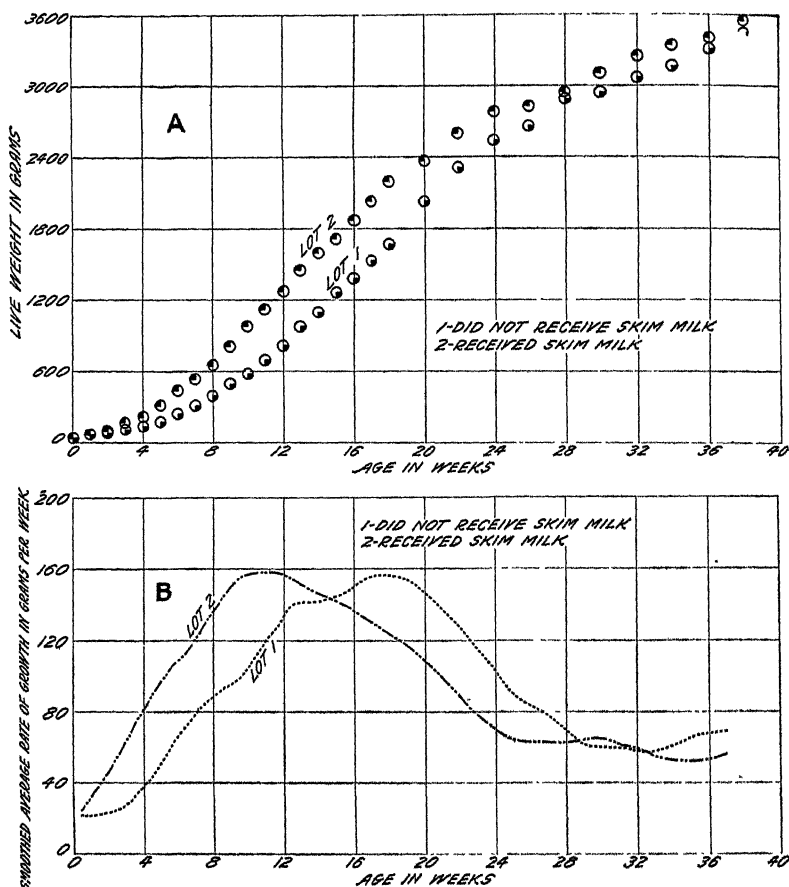


FIG. 10.—Comparison of the growth of the capons receiving skim milk with those not receiving it. A, cumulative growth curves; B, smoothed average-rate-of-growth curves

4 weeks of age in the cockerels receiving skim milk corresponded to 8.5 weeks of age in those not receiving it; that 5.8 weeks of age in the capons receiving skim milk corresponded to 9.3 weeks of age in those not receiving it, and that 4.2 weeks of age in the pullets receiving skim milk corresponded to 6.9 weeks of age in those not receiving it. It is to be noted that  $t^*$  for the capons was computed from data obtained after caponization and that the apparent discrepancy

<sup>11</sup> It should be noted that Brody's  $t^*$  is age from conception while the writers' is from birth (hatching).

between the differences in the two values for capons and for cockerels is probably due to this fact.

It is interesting to note that in the case of the lots of chickens receiving skim milk the point of inflection of the growth curve occurs earlier than in the case of those lots not receiving it; or, what appears to be the same thing, the whole of the growth curve of the former lots more nearly approaches the curve of diminishing increment than it does for the latter lots. Does this mean, then, that the more nearly a "perfect" condition of nutrition is reached the more nearly will the whole of the growth curve approach the curve of diminishing increment? This speculation is worthy of further consideration.

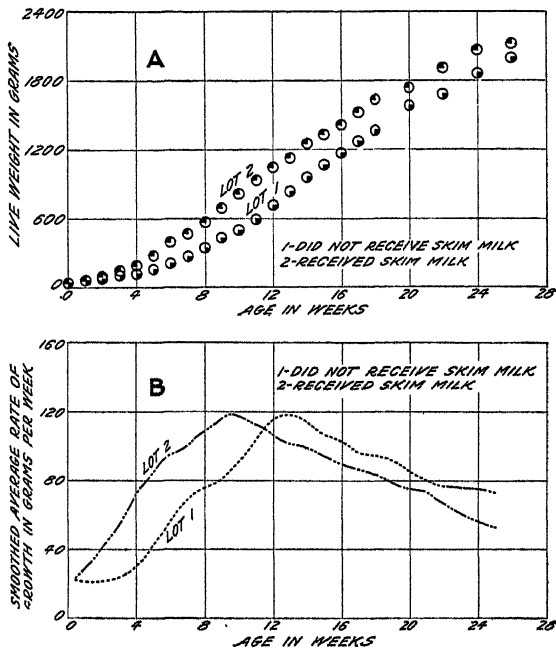


FIG. 11.—Comparison of the growth of the pullets receiving skim milk with those not receiving it. A, cumulative growth curves; B, smoothed average-rate-of-growth curves

#### THE INFLUENCE OF SEX ON THE GROWTH OF CHICKENS

It is a matter of common knowledge that the absolute rate of growth of the female chicken is less than that of the male and that its mature weight is less than that of the male. In the many records consulted the writers have not found a single exception to the above statement when the nutritional conditions were the same for both sexes. On this point the writers examined the growth data published by a number of investigators, including Bethke and Kennard (3), Buckner and his associates (8, 9), Card and Kirkpatrick (11), Jull (14), Kaupp (15), Kempster (16, 17), Latimer (18), Mitchell, Card, and Hamilton (20), and Philips (26).

In order to compare the rates of growth of the sexes, the writers have plotted together the smoothed average rates of growth of the

cockerels, capons, and pullets. (Fig. 12.) In the case of the lots receiving skim milk as well as in the case of the lots not receiving it, the rate of growth of the female is distinctly less than that of the male throughout the interval for which the writers obtained data on both sexes. According to the values of the constant A (Table 4) for the curve of diminishing increment, the mature female Rhode Island Red is on an average 1,108 gm. lighter than the male, or on a percentage basis, nearly 25 per cent lighter.

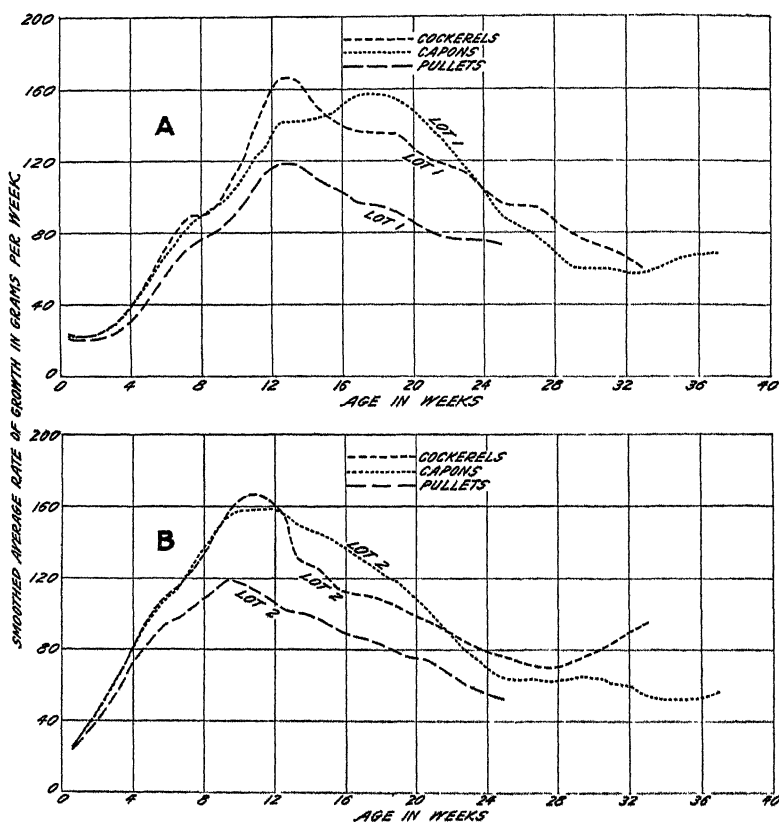


FIG. 12.—Comparison of the average-rate-of-growth curves for the different sexes. A, chickens not receiving skim milk; B, chickens receiving it

By means of the formula for the curve of diminishing increment one may calculate the time required for the two sexes to reach maturity (i. e., 98 per cent of the constant A, for, theoretically, A is never reached). Making the necessary calculations, the writers found that both the male and the female reach maturity (as defined above) at practically the same age, i. e., from 21 to 23 months. This is in fair agreement with the value obtained by Brody (5) for females of this breed, i. e., 25.2 months, or 25.9 months from the time the eggs were set.

## COCKERELS VERSUS CAPONS

Waite (31) has called attention to the fact "that the opinion is quite general among those interested in poultry, that capons make a much more rapid growth and attain a size nearly twice that of cockerels of the same age and breed." In order to obtain some definite data on the question of the relative growth of capons and cockerels, he conducted a rather carefully controlled experiment. After analyzing his results he found that there was practically no difference until the cockerels began to reach maturity, at which time the capons made slightly better gains. The breed used was the White Plymouth Rock.

In 1918 Philips (26) reported the results of a study on the cost of raising White Plymouth Rocks. He stated that capons and cockerels grew with similar rapidity and retained similar weights until they reached 6½ pounds and that cockerels made gains at less cost per pound for feed than pullets or capons.

Elford (12) compared rates of fattening and economy of gains of cockerels and capons, using 22 Barred Rock cockerels and 22 Barred Rock capons. His conclusion was that the value of caponizing lies rather in the production of meat of superior quality than in any greater efficiency of growth or fattening.

Mitchell, Card, and Hamilton's (20) rather comprehensive study of the growth of White Plymouth Rock chickens furnishes some comparative data on the growth of cockerels and capons. They found that the growth of the capon was not distinctly different from that of the cockerel. In a popular report of their work (21) they state that "cockerels and capons grew at nearly the same rate up to an average weight of 6 pounds, when the growth records were discontinued."

A confusion of the terms "growth" and "fattening" seems to be responsible for the different opinions held on this question. Under the usual conditions of rearing cockerels and capons, the capon seems to have a much greater tendency to fatten than the cockerel. This means that eventually the capon may become heavier than the cockerel but, as intimated, this is due to its greater tendency to fatten and not to any greater ability to grow. In fact, the writers' data seem to indicate that the capon's ability to grow, i. e., in the strict sense of the word, is somewhat lessened by the operation of caponizing.

The writers' data (Table 1) show that, in the case of Rhode Island Reds, the average cumulative growth of the two lots of cockerels was very similar to that of the two corresponding lots of capons; if anything, the cumulative growth of the latter, from the time of caponizing up to 34 weeks of age, was a little less than that of the former. In Figure 12 the smoothed average rate of growth of the cockerels is compared with that of the capons. Up to the time of caponizing (10 weeks) the average rate of growth of the lots designated as lot 1 cockerels, and lot 1 capons was similar as was also that of the lots designated as lot 2 cockerels and lot 2 capons. Comparing the average rate of growth of these lots after the caponizing, one finds that in the case of the birds not receiving skim milk the average rate of growth of the cockerels was greater than that of the capons up to the age of 15 weeks, less between the fifteenth and twenty-

fourth weeks, and greater for at least the next 9 weeks. In the case of the birds receiving skim milk the average rate of growth between the thirteenth and the twenty-second week was greater for the capons than the cockerels, but less from the twenty-second to at least the thirty-third week.

Comparing the values of  $A$  (Table 4) for the curve of diminishing increment for each lot of cockerels and capons, one finds that they are appreciably greater for the former than for the latter. These values of  $A$  indicate that the weight of the Rhode Island Red cockerel, when it matures, is approximately 4,459 gm., while that of the Rhode Island Red capon is only about 4,049 gm. This does not mean that the capon may not become heavier than the cockerel, for it may easily do so, owing to its greater tendency to fatten.

As already stated, the time required for cockerels and pullets to reach maturity was found to be from about 21 to 23 months. Similar calculations for the two lots of capons show that the time required is approximately the same for both lots and is 17.4 months. This, then, is one point in favor of the capon, i. e., that it matures nearly 5 months earlier than the cockerel.

Mitchell, Card, and Hamilton (21) found that in the protein content of gains put on, the cockerels ranked above the capons. This is in agreement with the writers' conclusion that so far as growth in its stricter sense is concerned, the cockerel is, if anything, superior to the capon.

#### SUMMARY

Growth data were obtained on two groups of Rhode Island Red chicks which received the same treatment and the same feed, with the one exception that one group had access to both sour skim milk and water while the other group received only water. Each group was composed of one lot each of cockerels, capons, and pullets.

A study of the variability of the growth data suggested to the writers that the variability of live weight, as indicated by the standard deviation, may be of some value in determining the approximate time of the beginning and end of growth cycles.

A period of high variability in live weight was observed in the case of each of the six lots. For the lots receiving skim milk this period was between 0 and 7 or 8 weeks of age and for the lots not receiving skim milk it was between 0 and at least 12 weeks of age.

The curve of a monomolecular, autocatalyzed, chemical reaction was fitted to the growth made by each lot during the first 15 to 18 weeks and the curve of diminishing increment was fitted to the later stages of the growth of each lot.

Most of the constants of each curve were markedly affected by the feeding of sour skim milk.

Feeding sour skim milk had no marked effect on the calculated mature weights.

The data indicate that milk feeding accelerated growth during the first 15 to 18 weeks; according to the smoothed average-rate-of-growth curves, this acceleration took place between the time of the first feeding and 11.5 to 14.5 weeks of age.

The data further show that the rate of growth of the chicks receiving no skim milk was somewhat greater during the later stages of growth than that of the chicks receiving it.

The constant  $R$  (or  $\epsilon - k$ ) for the curve of diminishing increment was not affected by feeding milk; its value was rather constant for each of the six lots.

The calculated average-rate-of-growth curves for the first 15 to 18 weeks were found to be narrower and higher for the lots receiving skim milk than for the lots not receiving it.

In the case of the lots of chicks receiving skim milk the point of inflection of the growth curve occurred earlier than in the case of the lots not receiving it. This suggested to the writers that the more nearly a "perfect" condition of nutrition is approached the more nearly will the whole of the growth curve approach the curve of diminishing increment.

The mature weights of Rhode Island Red cockerels, capons, and pullets were found to be, according to the values of the constant  $A$  for the curve of diminishing increment, 4,459, 4,049, and 3,351 gm., respectively.

By means of the equation of the curve of diminishing increment it was estimated that the ages at which maturity is reached are 21 to 23 months for cockerels and pullets and 17.4 months for capons.

#### LITERATURE CITED

- (1) ANDERSON, W. B.  
1898. SKIM MILK AS FOOD FOR YOUNG GROWING CHICKENS. Ind. Agr. Expt. Sta. Bul. 71: 60-63.
- (2) ———  
1899. SKIM MILK AS A FOOD FOR YOUNG GROWING CHICKENS. Ind. Agr. Expt. Sta. Bul. 76: [21]-28.
- (3) BETHKE, R. M., and KENNARD, D. C.  
1924-25. REARING CHICKENS TO MATURITY INDOORS. Poultry Sci. 4: 74-78, illus.
- (4) BRODY, S.  
1921. THE RATE OF GROWTH OF THE DOMESTIC FOWL. Jour. Gen. Physiol. 3: 765-770, illus.
- (5) ———  
1926. TIME RELATIONS OF GROWTH. I. GENETIC GROWTH CONSTANTS OF ANIMALS. Jour. Gen. Physiol. 8: 233-251, illus.
- (6) ———  
1927. TIME RELATIONS OF GROWTH. III. GROWTH CONSTANTS DURING THE SELF-ACCELERATING PHASE OF GROWTH. Jour. Gen. Physiol. 10: 637-658, illus.
- (7) ——— SPARROW, C. D., and KIBLER, H. H.  
1926. TIME RELATIONS OF GROWTH. II. THE EQUIVALENCE OF AGE IN MAMMALS ESTIMATED ON THE BASIS OF THEIR GROWTH CONSTANTS. Jour. Gen. Physiol. 9: 285-308, illus.
- (8) BUCKNER, G. D., NOLLAU, E. H., WILKINS, R. H., and KASTLE, J. H.  
1919. EFFECT OF CERTAIN GRAIN RATIONS ON THE GROWTH OF THE WHITE LEGHORN CHICK. Jour. Agr. Research 16: 305-312, illus.
- (9) ——— WILKINS, R. H., and KASTLE, J. H.  
1918. THE NORMAL GROWTH OF WHITE LEGHORN CHICKENS. Amer. Jour. Physiol. 47: 393-398, illus.
- (10) CARD, L. E.  
1926. A COMPARISON OF DIFFERENT FORMS OF MILK IN A RATION FOR GROWING CHICKS. Poultry Sci. 5: 199-202, illus.
- (11) ——— and KIRKPATRICK, W. F.  
1918. REARING CHICKENS. PART I. NORMAL RATE OF GROWTH IN WHITE LEGHORNS AND RHODE ISLAND REDS. Conn. Storrs Agr. Expt. Sta. Bul. 96: [355]-372, illus.
- (12) ELFORD, F. C.  
1924. [POULTRY INVESTIGATIONS AT THE DOMINION EXPERIMENTAL FARMS]. Canada Expt. Farms, Poultry Div. Rpt. 1923: 10-31, illus.

- (13) GOODALE, H. D.  
1926. EARLY GROWTH RATES OF CHICKENS WITH SPECIAL REFERENCE TO ULTRA-VIOLET LIGHT. *Amer. Jour. Physiol.* 79: 44-60, illus.
- (14) JULL, M. A.  
1923. DIFFERENTIAL SEX GROWTH CURVES IN BARRED PLYMOUTH ROCK CHICKS. *Sci. Agr.* 4: 58-65, illus.
- (15) KAUPP, B. F.  
1921-22. THE RATE OF GROWTH OF SINGLE COMB RHODE ISLAND REDS. *Poultry Sci.* 1: [39]-43, illus.
- (16) KEMPSTER, H. L., and HENDERSON, E. W.  
1923. RATE OF GROWTH OF THE DOMESTIC FOWL. *Missouri Agr. Expt. Sta. Research Bul.* 62: 40-52, illus.
- (17) ——— and SEATON, M. A.  
1926. POULTRY HUSBANDRY. *Missouri Agr. Expt. Sta. Bul.* 236: 64-67.
- (18) LATIMER, H. B.  
1925. POSTNATAL GROWTH OF THE BODY, SYSTEMS, AND ORGANS OF THE SINGLE-COMB WHITE LEGHORN CHICKEN. *Jour. Agr. Research* (1924) 29: 363-397, illus.
- (19) LEWIS, H. R.  
1919. PROTECTIVE FEEDS FOR POULTRY. *N. J. Agr. Expt. Sta. Hints to Poultrymen*, v. 7, no. 7, [4] p.
- (20) MITCHELL, H. H., CARD, L. E., and HAMILTON, T. S.  
1926. THE GROWTH OF WHITE PLYMOUTH ROCK CHICKENS. *Ill. Agr. Expt. Sta. Bul.* 278, p. 71-132, illus.
- (21) ——— CARD, L. E., and HAMILTON, T. S.  
1927. HOW CHICKENS GROW. *Natl. Poultry Jour.* [U. S.] 5 (7): 7.
- (22) MURRAY, H. A., Jr.  
1926. PHYSIOLOGICAL ONTOGENY. A. CHICKEN EMBRYOS. VIII. ACCELERATIONS OF INTEGRATION AND DIFFERENTIATION DURING THE EMBRYONIC PERIOD. *Jour. Gen. Physiol.* 9: 603-619, illus.
- (23) NEW YORK [STATE] AGRICULTURAL EXPERIMENT STATION.  
1892. FEEDING EXPERIMENTS WITH POULTRY. SKIM-MILK FOR GROWING CHICKS. *N. Y. State Agr. Expt. Sta. Bul.* (n. s.) 39: [11]-15.
- (24) ORR, J. B., ROBERTSON, G. S., KINROSS, A., LEWIS, G., and NEWBIGGIN, H.  
1926. THE NUTRITIVE REQUIREMENTS OF POULTRY. VII. NOTE ON GROWTH IN CHICKENS. (PRELIMINARY COMMUNICATION.) *Scot. Jour. Agr.* 9: 392-395.
- (25) OSBORNE, T. B., and MENDEI, L. B.  
1916. THE EFFECT OF THE AMINO-ACID CONTENT OF THE DIET ON THE GROWTH OF CHICKENS. *Jour. Biol. Chem.* 26: 293-300, illus.
- (26) PHILIPS, A. G.  
1918. COST OF RAISING WHITE PLYMOUTH ROCKS. *Ind. Agr. Expt. Sta. Bul.* 214, 23 p., illus.
- (27) RETTGER, L. F., KIRKPATRICK, W. F., and CARD, L. E.  
1915. CHICKENS: MILK FEEDING AND ITS INFLUENCE ON GROWTH AND MORTALITY. COMPARATIVE STUDY OF THE VALUE OF SWEET AND SOUR MILK. *Conn. Storrs Agr. Expt. Sta. Bul.* 80, 28 p., illus.
- (28) ROBERTSON, T. B.  
[1923]. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. (Monographs on Experimental Biology.)
- (29) SANDE-BAKHUYZEN, H. L. VAN DE  
1926. GROWTH AND GROWTH FORMULAS IN PLANTS. *Science* (n. s.) 64: 653-654.
- (30) ——— and ALSBERG, C. L.  
1927. THE GROWTH CURVE IN ANNUAL PLANTS. *Physiol. Rev.* 7: 151-187.
- (31) WAITE, R. H.  
1920. CAPONS VS. COCKERELS—A COMPARISON OF THEIR RATE OF GROWTH AND FEED CONSUMPTION. *Md. Agr. Expt. Sta. Bul.* 235, p. 119-132, illus.
- (32) WHEELER, W. P.  
1899. ANIMAL FOOD FOR POULTRY. *N. Y. State Agr. Expt. Sta. Bul.* 171, p. [461]-506, illus.



# GROWTH OF CHICKENS IN RELATION TO FEED CONSUMPTION<sup>1</sup>

By MORLEY A. JULL, *Poultry Husbandman*, and HARRY W. TITUS, *Associate Biological Chemist, Bureau of Animal Industry, United States Department of Agriculture*.

## RELATION BETWEEN GROWTH AND FEED CONSUMPTION

The rate at which an animal producing flesh for human consumption gains in weight, as expressed by the number of units of feed required for the successive unit increases in live weight, is a problem of considerable practical importance. In the raising of poultry for market it is well recognized that beyond a certain stage in growth the value of the feed required to produce 1 pound gain in weight of live animal is out of all proportion to the value of the gain. Hence there has developed the common practice of marketing growing poultry at from 8 weeks to about 8 months of age. So far as the authors are aware, however, no experimental work has ever been undertaken to determine precisely the relationship between growth and feed consumption in the domestic fowl.

In poultry husbandry there are several types of phenomena which involve a gradual decrease in the per unit effect of some causal factor as the quantity of the factor acting increases. One phenomenon illustrating this point is the annual decrease in the egg production of hens, which after three or four years is so great that few hens produce eggs enough to justify the feed consumed. At the United States Animal Husbandry Experiment Farm, Beltsville, Md., it was found that for a period of three years, with a small group of birds, the annual average egg production per bird was 236, 188, and 167 for the first, second, and third years, respectively. Egg production decreased each year, and assuming that laying hens consume approximately the same amounts of feed each succeeding year, it is obvious that egg production would soon become unprofitable. Brody, Henderson, and Kempster,<sup>2</sup> in a rather large group of birds of two breeds, observed that each year's production was 88 per cent of that of the preceding year.

Another illustration of gradual decrease in the per unit effect of some causal factor as the quantity of the factor acting increases, may be cited in the results obtained at the United States Animal Husbandry Experiment Farm in feeding laying hens different quantities of protein. In rations in which the protein, except that contained in the grains, was provided exclusively from meat scraps, egg production increased as the protein level was raised, but above a protein level of 20 per cent egg production did not increase at the same rate as the protein level was increased. In other words, as the protein level was increased above the 20 per cent level there was relatively a decreasing rate of egg production.

<sup>1</sup> Received for publication Jan. 27, 1928; issued May 1, 1928.

<sup>2</sup> BRODY, S., HENDERSON, E. W., and KEMPSTER, H. L. THE RATE OF SENESCENCE OF THE DOMESTIC FOWL AS MEASURED BY THE DECLINE IN EGG PRODUCTION WITH AGE. *Jour. Gen. Physiol.* 6: 41-45, illus. 1923.

In the growing of chickens the same principle applies; there comes a time when the rate of growth decreases although feed consumption may be on the increase. Available data indicate that the relationship between increase in live weight in growing chickens and increase of feed consumed is expressed by the law of diminishing increment. Though comparatively few data are available to test the application of the law in the case of growing chickens, a rather complete discussion of it in its application to cattle, hogs, and chickens is given by Spillman.<sup>3</sup> A very interesting feature of the law is that it provides for the calculation of the rate of gain per feed unit, and the number of feed units per pound of gain, in growing chickens and in chickens that are being fattened for market. The purpose of the present experiment was to provide data, obtained under controlled conditions, to test the application of the law of diminishing increment as applied to growing chickens.

#### EXPERIMENTAL PROCEDURE

On April 24, 1925, there were hatched 170 chicks from a pen of Barred Plymouth Rock females mated to Rhode Island Red males. This mating was used in order to make possible the separation of the sexes at hatching time, there being involved the sex-linked barring factor of the females which was transmitted to the male chicks only. The male chicks had a white patch on the back of the head and also had yellow shanks, whereas the female chicks were without the white spot and had black shanks.

Of the 170 chicks, 84 were females and 86 were males. The females were divided into lot 1 with 40 chicks and lot 2 with 44 chicks. The males were divided into lot 3 with 43 chicks and lot 4 with 43 chicks. During the first three days there were 7 deaths in lot 1, 3 in lot 2, 0 in lot 3, and 3 in lot 4. Therefore, the number of chicks in each lot on which data were obtained was: Lot 1, 33 females; lot 2, 41 females; lot 3, 43 males; and lot 4, 40 males.

All lots were treated as uniformly as possible in respect to brooding and management. Each lot was brooded under an electric brooder in a pen 4 feet by 15 feet which adjoined a concrete yard 4 feet by 8 feet. At 8 weeks of age each lot was moved to a colony house 5 feet by 6 feet in a yard 100 feet by 200 feet and kept there until the conclusion of the experiment.

The four lots received the same ration and were fed in the same manner. During the first four weeks commercial chick feed was fed once daily and dry mash was fed twice daily, the mash being left before the chicks in hoppers for one hour each feeding time. After the first four weeks a scratch ration of 2 parts, by weight, cracked corn and 1 part wheat was fed twice daily and dry mash was kept in self-feeding hoppers before the birds at all times. The amount of scratch feed given daily was determined by the appetites of the birds, only such quantities being fed each day as would be eaten within a few minutes after being fed. The dry mash from

<sup>3</sup> SPILLMAN, W. J., and LANG, E. *THE LAW OF DIMINISHING RETURNS*. 178 p., illus. Chicago, World Book Co. 1924.

hatching time to the conclusion of the experiment was composed of the following parts by weight: Corn meal 40, sifted crushed oats 40, bran 20, middlings 20, beef scraps 10, dried buttermilk 10, bone meal 5, chick oyster shell 4, and cod-liver oil 3. The birds had free access to grit and water at all times.

Each chick was weighed separately at hatching time and every two weeks thereafter, the weights being recorded in grams to the second decimal place. The total amount of feed consumed by each lot was determined at the end of every two-week period.

The mortality throughout the duration of the experiment was comparatively low, one bird in each lot dying on the dates given: Lot 1, May 18 and July 17; lot 2, no deaths; lot 3, June 8 and 11, July 17 and 31; lot 4, May 20, July 16, and September 23.

#### AVERAGE WEIGHTS AND FEED CONSUMPTION

Table 1 gives the mean or average weight per chick in each of the four lots at hatching time and at the end of each two-week period thereafter. It will be observed that for the first 6 or 8 weeks the females weighed practically the same as the males, but that after 10 weeks the males increased in weight more rapidly than the females. A similar phenomenon was observed by Jull<sup>4</sup> in the case of Barred Plymouth Rock male and female chicks and by Titus and Jull<sup>5</sup> in the case of Rhode Island Red male and female chicks.

TABLE 1.—*The mean (average) weight per chick in each of the four lots at hatching time and each two-week period thereafter*

Age (in weeks)	Mean weight per chick in—				Age (in weeks)	Mean weight per chick in—			
	Lot 1 (females)	Lot 2 (females)	Lot 3 (males)	Lot 4 (males)		Lot 1 (females)	Lot 2 (females)	Lot 3 (males)	Lot 4 (males)
	Grams	Grams	Grams	Grams		Grams	Grams	Grams	Grams
0.....	34.54	34.19	34.97	34.77	14.....	1,334.55	1,236.59	1,505.87	1,629.74
2.....	90.82	79.76	77.12	82.27	16.....	1,541.29	1,480.37	1,772.95	1,933.03
4.....	215.31	193.10	185.44	225.59	18.....	1,651.77	1,574.15	2,046.03	2,157.37
6.....	337.25	355.76	366.77	399.43	20.....	1,860.16	1,785.43	2,386.79	2,435.55
8.....	618.13	532.71	608.10	656.59	22.....	2,026.93	2,012.07	2,736.41	2,744.86
10.....	835.94	770.85	773.17	924.23	24.....	2,211.45	2,170.97	2,906.28	2,926.89
12.....	985.45	936.95	1,061.10	1,225.66					

Table 2 gives the various statistical constants for the mean weights shown in Table 1. The values for the coefficients of variation show that the live weights of the birds in lot 1 (females) were most variable during the first two weeks after which they steadily became more uniform, but that in lots 2 (females), 3 (males), and 4 (males) the variability in live weight increased up to six weeks, decreasing almost steadily thereafter.

<sup>4</sup> JULL, M. A. DIFFERENTIAL SEX GROWTH CURVES IN BARRED PLYMOUTH ROCK CHICKS. *Sci. Agr.* 4: 58-65, illus. 1923.

<sup>5</sup> TITUS, H. W., and JULL, M. A. THE GROWTH OF RHODE ISLAND REDS AND THE EFFECT OF FEEDING SKIM MILK ON THE CONSTANTS OF THEIR GROWTH CURVES. *Jour. Agr. Research* 36: 515-540, illus. 1923.

TABLE 2.—*The probable error of the mean weights per chick for each of the four lots together with the standard deviation and coefficient of variation of each mean weight*

Age (in weeks)	Statistical constants	Lot 1 (females)	Lot 2 (females)	Lot 3 (males)	Lot 4 (males)
0	P. E. of mean.....gm.	± 0.24	± 0.24	± 0.22	± 0.23
	σ.....gm.	2.02± .17	2.32± .17	2.15± .16	2.16± .16
	C. of V.....per cent.	5.85± .48	6.78± .50	6.15± .45	6.21± .47
2	P. E. of mean.....gm.	± 1.88	± 1.32	± 1.56	± 1.50
	σ.....gm.	16.00± 1.33	12.56± .93	15.21± 1.11	14.06± 1.06
	C. of V.....per cent.	17.62± 1.51	15.75± 1.20	19.72± 1.49	17.09± 1.33
4	P. E. of mean.....gm.	± 4.20	± 3.52	± 4.22	± 4.61
	σ.....gm.	35.26± 2.97	33.44± 2.49	41.04± 2.98	42.73± 3.26
	C. of V.....per cent.	16.38± 1.42	16.88± 1.31	22.13± 1.69	18.94± 1.50
6	P. E. of mean.....gm.	± 6.71	± 7.18	± 9.75	± 8.20
	σ.....gm.	56.25± 4.74	68.19± 5.08	94.77± 6.89	75.92± 5.80
	C. of V.....per cent.	14.52± 1.25	17.28± 1.33	25.84± 2.00	19.01± 1.50
8	P. E. of mean.....gm.	± 9.26	± 9.04	± 15.07	± 12.39
	σ.....gm.	77.68± 6.55	85.86± 6.39	143.05± 10.65	114.70± 8.76
	C. of V.....per cent.	12.57± 1.08	14.73± 1.12	23.52± 1.85	17.47± 1.37
10	P. E. of mean.....gm.	± 12.57	± 11.22	± 19.43	± 15.74
	σ.....gm.	105.39± 8.88	106.55± 7.94	184.47± 13.74	145.74± 11.13
	C. of V.....per cent.	12.61± 1.08	13.82± 1.05	23.86± 1.88	15.77± 1.23
12	P. E. of mean.....gm.	± 14.89	± 11.56	± 19.54	± 17.54
	σ.....gm.	122.91± 10.53	109.73± 8.17	183.26± 13.82	160.31± 12.40
	C. of V.....per cent.	12.47± 1.08	11.71± .88	16.79± 1.30	13.08± 1.03
14	P. E. of mean.....gm.	± 17.61	± 12.43	± 24.84	± 20.03
	σ.....gm.	145.36± 12.45	118.03± 8.79	232.93± 17.57	183.03± 14.16
	C. of V.....per cent.	10.89± .94	9.54± .71	15.47± 1.19	11.23± .88
16	P. E. of mean.....gm.	± 19.79	± 13.87	± 25.24	± 19.55
	σ.....gm.	163.37± 13.99	131.67± 9.81	233.64± 17.84	178.71± 13.83
	C. of V.....per cent.	10.60± .92	8.89± .66	13.18± 1.03	9.24± .71
18	P. E. of mean.....gm.	± 20.64	± 13.91	± 25.78	± 20.76
	σ.....gm.	170.38± 14.59	132.10± 9.84	238.68± 18.23	189.72± 14.68
	C. of V.....per cent.	10.31± .89	8.39± .62	11.62± .90	8.79± .68
20	P. E. of mean.....gm.	± 21.68	± 19.69	± 27.64	± 20.98
	σ.....gm.	178.96± 15.33	186.00± 13.85	255.90± 19.54	191.76± 14.84
	C. of V.....per cent.	9.62± .82	9.27± .61	10.72± .83	7.87± .61
22	P. E. of mean.....gm.	± 26.48	± 23.06	± 30.70	± 26.82
	σ.....gm.	218.54± 18.72	218.88± 16.30	284.16± 21.70	241.83± 18.96
	C. of V.....per cent.	10.78± .93	10.88± .82	10.38± .80	8.81± .69
24	P. E. of mean.....gm.	± 32.59	± 26.24	± 31.90	± 28.65
	σ.....gm.	269.01± 23.04	249.14± 18.56	295.31± 22.55	258.35± 20.26
	C. of V.....per cent.	12.16± 1.06	11.47± .87	10.16± .78	8.83± .69

Table 3 shows the feed consumption in grams per chick for each two-week period for each of the four lots.

TABLE 3.—*Average feed consumption per chick for each two-week period*

Age (in weeks)	Average feed consumption per chick				Age (in weeks)	Average feed consumption per chick			
	Lot 1 (females)	Lot 2 (females)	Lot 3 (males)	Lot 4 (males)		Lot 1 (females)	Lot 2 (females)	Lot 3 (males)	Lot 4 (males)
	Grams	Grams	Grams	Grams		Grams	Grams	Grams	Grams
2	127.14	132.76	111.82	136.08	16	1,251.04	1,063.18	1,284.02	1,309.45
4	271.16	246.16	236.29	257.39	18	1,316.88	1,231.34	1,639.91	1,647.26
6	511.28	472.40	489.99	536.40	20	1,534.90	1,205.89	1,520.70	1,715.89
8	688.19	677.62	788.26	814.14	22	1,559.77	1,416.09	1,721.33	1,745.11
10	846.94	824.76	863.49	1,116.54	24	1,613.18	1,261.21	1,625.37	1,764.11
12	1,176.41	1,045.48	1,219.03	1,287.96					
14	1,256.89	1,080.88	1,322.22	1,406.14	Total	12,153.78	10,657.77	12,822.43	13,736.47

APPLICATION OF THE LAW OF DIMINISHING INCREMENT

In order to illustrate the law of diminishing increment graphically the observed average weights of the birds in lot 1 have been plotted as ordinates and the values of the cumulative feed consumption per bird have been plotted as abscissas in Figure 1. The smooth curve passing through the plotted points is the curve of diminishing increment fitted to the last eight of these points. It is readily apparent from the graph that for each successive 1,000 gm. of feed consumed there is a proportionately smaller increase in live weight.

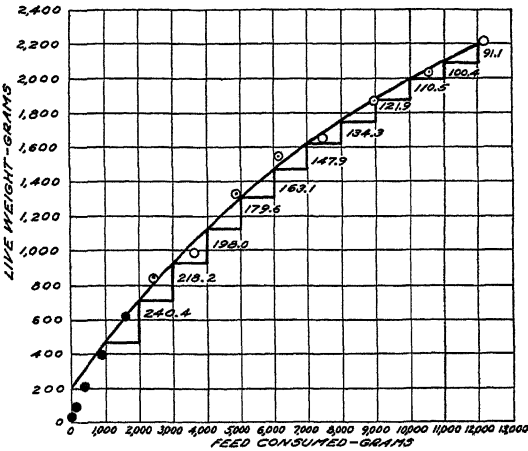


FIG. 1.—Graphic illustration of the law of diminishing increment in chickens; based on the data for lot 1

The number of grams in live weight is shown for each 1,000 gm. of feed consumed, and by calculation it is found that for each successive 1,000 gm. of feed consumed the increase in live weight is approximately 90.7 per cent of the preceding increase in live weight. From the graph one may also read the number of grams of feed required to produce any given average live weight, above 600 gm., of the birds in lot 1.

Table 4 shows for each of the four lots, (1) the average feed consumption per bird per two-week period; (2) the average total feed consumption per bird; (3) the observed average live weight of the birds; and (4) the calculated average live weight of the birds.

TABLE 4.—Relation between feed consumption and live weight of chickens in lots # to 4

LOT 1 (FEMALES)

Age (in weeks)	Number of birds	Average feed consumption		Average weight per bird		Differences*
		Per period	Total	Observed	Calculated*	
		Grams	Grams	Grams	Grams	
0.....	33			34.5		
2.....	32	127.14	127.1	90.8		
4.....	32	271.16	398.3	215.3		
6.....	32	511.28	909.6	387.3		
8.....	32	688.19	1,597.8	618.1		
10.....	32	846.94	2,444.7	835.9	814.1	+21.8
12.....	31	1,176.41	3,621.1	985.5	1,057.8	-72.3
14.....	31	1,256.89	4,878.0	1,394.6	1,269.2	+125.4
16.....	31	1,251.04	6,129.1	1,541.3	1,493.2	+48.1
18.....	31	1,316.88	7,445.9	1,651.8	1,632.7	+19.1
20.....	31	1,534.90	8,980.8	1,860.2	1,875.1	-14.9
22.....	31	1,559.77	10,540.6	2,026.9	2,043.4	-16.5
24.....	31	1,613.18	12,153.8	2,211.5	2,192.6	+18.9
Root-mean-square deviation.....						±38.5

\* In fitting the curve of diminishing increment only the data obtained after the eighth week were used

TABLE 4.—*Relation between feed consumption and live weight of chickens in lots 1 to 4—Continued*

## · LOT 2 (FEMALES)

Age (in weeks)	Number of birds	Average feed consumption		Average weight per bird		Difference
		Per period	Total	Observed	Calculated	
		Grams	Grams	Grams	Grams	Grams
0.....	41			34.2		
2.....	41	132.76	132.8	79.8		
4.....	41	246.16	378.9	198.1		
6.....	41	472.40	851.3	355.8		
8.....	41	677.62	1,528.9	582.7		
10.....	41	824.76	2,353.7	770.9	756.8	+14.1
12.....	41	1,045.48	3,399.2	937.0	990.4	-53.4
14.....	41	1,080.88	4,480.1	1,236.6	1,211.9	+24.7
16.....	41	1,063.18	5,543.2	1,480.4	1,411.4	+69.0
18.....	41	1,231.34	6,774.6	1,574.2	1,621.8	-47.6
20.....	41	1,205.89	7,980.5	1,785.4	1,808.3	-22.9
22.....	41	1,416.09	9,396.6	2,012.1	2,005.1	+7.0
24.....	41	1,261.21	10,657.8	2,171.0	2,162.2	+8.8
Root-mean-square deviation.....						±37.6

## LOT 3 (MALES)

0.....	43			35.0		
2.....	43	111.82	111.8	77.1		
4.....	43	236.29	348.1	185.4		
6.....	43	489.99	838.1	366.8		
8.....	41	788.26	1,626.3	608.1		
10.....	41	863.49	2,489.8	773.2	773.2	
12.....	40	1,219.03	3,708.9	1,091.1	1,116.9	-25.8
14.....	40	1,322.22	5,031.1	1,505.9	1,457.6	+48.3
16.....	39	1,284.02	6,315.1	1,773.0	1,759.2	+13.8
18.....	39	1,639.91	7,955.0	2,046.0	2,106.3	-60.3
20.....	39	1,520.70	9,475.7	2,386.8	2,394.0	-7.2
22.....	39	1,721.33	11,197.0	2,736.4	2,684.3	+52.1
24.....	39	1,625.37	12,822.4	2,906.3	2,927.6	-21.3
Root-mean-square deviation.....						±35.4

## LOT 4 (MALES)

0.....	40			34.8		
2.....	40	136.08	136.1	82.3		
4.....	39	257.39	393.5	225.6		
6.....	39	536.40	929.9	399.4		
8.....	39	814.14	1,744.0	656.6		
10.....	39	1,116.54	2,860.5	924.2	915.9	+8.3
12.....	38	1,287.96	4,148.5	1,225.7	1,264.9	-39.2
14.....	38	1,406.14	5,554.6	1,629.7	1,603.6	+26.1
16.....	38	1,309.45	6,864.1	1,933.0	1,883.7	+49.3
18.....	38	1,647.26	8,511.3	2,157.4	2,193.6	-36.2
20.....	38	1,715.89	10,237.2	2,435.5	2,472.2	-36.7
22.....	37	1,745.11	11,972.3	2,744.9	2,715.9	+29.0
24.....	37	1,764.11	13,736.5	2,926.9	2,927.2	-.3
Root-mean-square deviation.....						±32.0

Table 5 shows, for each of the four lots, the expected live weight for each 1,000 gm. of feed consumed. The values in the sixth column of Table 4 and for each of the lots in Table 5 were calculated by means of the formula of the curve of diminishing increment fitted to the data obtained after the eighth week.

TABLE 5.—*The calculated average weight of the birds in each lot per 1,000 gm. cumulative feed consumption per bird*

Average total feed consumption per bird (in grams)	Calculated average weight of the birds				Average total feed consumption per bird (in grams)	Calculated average weight of the birds			
	Lot 1	Lot 2	Lot 3	Lot 4		Lot 1	Lot 2	Lot 3	Lot 4
	Grams	Grams	Grams	Grams		Grams	Grams	Grams	Grams
1,000.....	474.0	423.1	310.6	337.1	8,000.....	1,755.5	1,811.2	2,115.3	2,102.1
2,000.....	714.4	673.1	626.5	659.8	9,000.....	1,877.4	1,952.3	2,307.3	2,277.2
3,000.....	932.6	903.6	920.7	955.6	10,000.....	1,987.9	2,082.3	2,486.2	2,437.7
4,000.....	1,130.6	1,115.9	1,194.5	1,226.6	11,000.....	2,088.3	2,202.1	2,652.8	2,584.7
5,000.....	1,310.2	1,311.6	1,448.9	1,474.9	12,000.....	2,179.4	2,312.5	2,807.9	2,719.4
6,000.....	1,473.3	1,491.9	1,687.7	1,702.5	13,000.....	2,262.0	2,414.3	2,952.6	2,842.9
7,000.....	1,621.2	1,658.1	1,909.1	1,911.0	14,000.....	2,337.0	2,508.0	3,087.1	2,956.0

The method used in fitting the curve of diminishing increment to the writers' experimental data is essentially different from the methods described by Spillman <sup>6</sup> and for that reason is given in some detail here. No originality is claimed for this method; it is a combination of methods which have been used by others. The general formula for the curve of diminishing increment, as written by Spillman, is

$$Y = M - AR^x \text{-----} (1)$$

in which

- Y=live weight for any given amount, *x*, of feed consumed.
- M=theoretical maximum live weight attainable,
- A=the gain in live weight, above the *theoretical* initial weight, of which the birds are capable if their growth continues to obey the same law,
- R=ratio between gains in live weight for successive units of feed, and
- x*=the number of units of feed consumed.

The first step is to transform the equation into its linear form, which is:

$$\log A + x \log R - \log (M - Y) = 0 \text{-----} (2)$$

If one now selects a value for M and plots log (M - Y) against *X*, an approximately straight line results, if the selected value of M is sufficiently close to its true value. By trying several different values for M, the true value may thus be approximated. Having obtained an approximate value of M, the next step is to substitute it in equation (2) and then determine the values of A and R by the method of least squares. (Figs. 2 to 5.)

Having approximate values for M, A, and R, one is now ready to "adjust" them by the usual method.

The authors have found that the computations incident to making these adjustments are very much simplified if equation (1) is re-written as follows:

$$Y = M - Ae^{-kx} \text{-----} (3)$$

<sup>6</sup> SPILLMAN, W. J., and LANG, E. Op. cit.

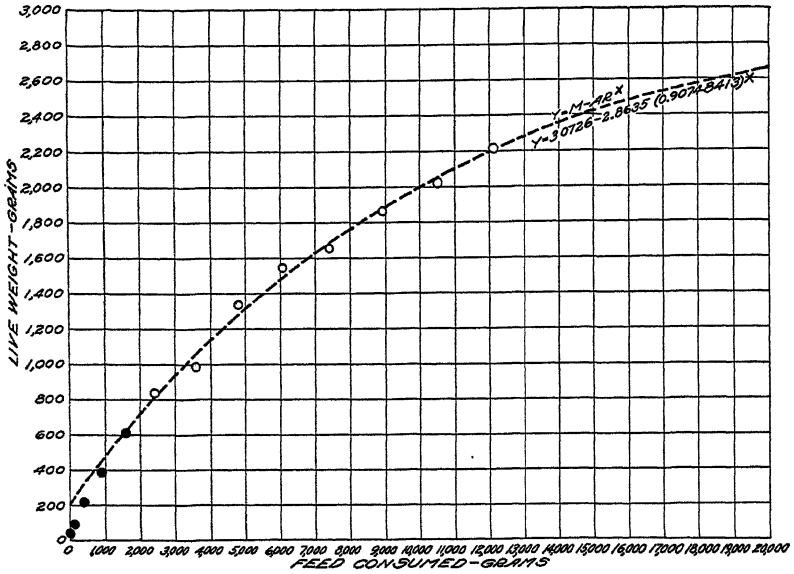


FIG. 2.—Relation between weight of birds in lot 1 (females) and average amount of feed consumed

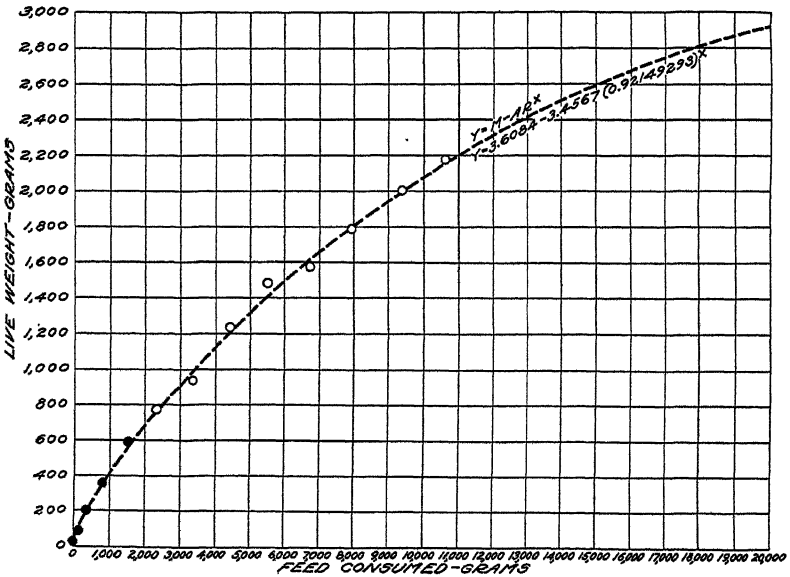


FIG. 3.—Relation between weight of birds in lot 2 (females) and average amount of feed consumed



in which  $\epsilon^{-k}$  replaces  $R$  of equation (1). The "adjustment" equation for this form of the equation of the curve of diminishing increment is:

$$\frac{\delta f}{\delta M_0} \mu + \frac{\delta f}{\delta A_0} \alpha + \frac{\delta f}{\delta k_0} \kappa = Y - Y_0 \text{-----} (4)$$

in which

$$\begin{aligned} \frac{\delta f}{\delta M_0} &= 1, \\ \frac{\delta f}{\delta A_0} &= \epsilon^{-k_0}, \\ \frac{\delta f}{\delta k_0} &= x A_0 \epsilon^{-k_0 x}. \end{aligned}$$

$\mu$ ,  $\alpha$ , and  $\kappa$  are corrections to be made to  $M_0$ ,  $A_0$  and  $k_0$ , respectively, which are the approximate values of  $M$ ,  $A$  and  $R$  previously obtained, and  $Y$  and  $Y_0$  are, respectively, the observed and calculated values of  $Y$ .

By readjusting the corrected values of  $M$ ,  $A$ , and  $k$  until the corrections become negligible (two to three adjustments were found to be necessary), one finally obtains with considerable accuracy the most probable values of these constants. It is now a simple matter to calculate the most probable value of  $R$  from the relationship between  $R$  and  $k$ , that is,  $R = \epsilon^{-k}$ .

The above method was applied to each of the four sets of data, and the values of the constants shown in Table 6 were obtained.

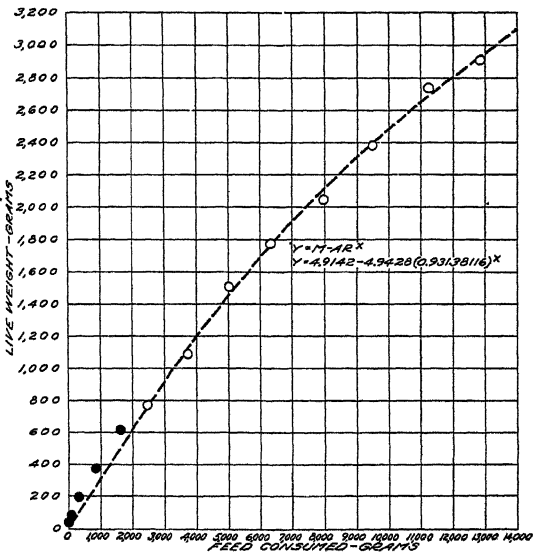


FIG. 4.—Relation between weight of birds in lot 3 (males) and average amount of feed consumed

TABLE 6.—*Constants of the curve of diminishing increment* <sup>a</sup>

Lot	M	A	R	k <sup>b</sup>	S <sup>c</sup>
	Kilograms	Kilograms			Kilogram
1, females.....	3. 0726	2. 8635	0. 907484	0. 0970791	0. 0385
2, females.....	3. 6084	3. 4567	. 921493	. 0817604	. 0376
3, males.....	4. 9142	4. 9428	. 931381	. 0710867	. 0354
4, males.....	4. 1954	4. 2105	. 916351	. 0873561	. 0320

<sup>a</sup> The unit of live weight, as well as the unit of feed weight used in determining these constants, was the kilogram; the same unit should be used in making any calculations involving these constants.

<sup>b</sup> See equation (3).

<sup>c</sup> S is the root-mean-square deviation of the observed values of  $Y$  from the calculated values of  $Y$ .

The differences between the observed and calculated values of the average live weights for each of the four lots (column 7, of Table 4), were found to be relatively small. It was also found that the root-mean-square deviation of the calculated from the observed live weights was of the same order of magnitude for each of the four lots.

It is rather difficult to explain the difference between the values of  $M$  for lots 1 and 2, as well as for lots 3 and 4, since these were duplicate lots which received the same treatment throughout. The same may be said of the values of  $A$ . Tables 1 and 4 show, however, that these duplicate lots were not strictly comparable<sup>7</sup> at the tenth week; and it was to the data obtained from the tenth to the twenty-fourth week that the curve of diminishing increment was fitted.

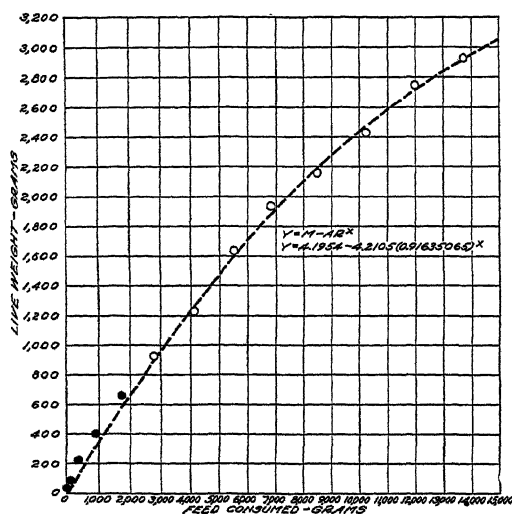


FIG. 5.—Relation between weight of birds in lot 4 (males) and average amount of feed consumed

to reach that weight. In any case, the values of  $R$  indicate that, for the feed interval to which the curve of diminishing increment was fitted, the ratio between gains in live weight for successive kilogram units of feed is approximately 0.92. In view of the agreement between the observed and calculated live weights, the writers are led to the conclusion that the relationship between feed consumption and growth of the domestic fowl is expressible by the law of diminishing increment, at least for the feed interval studied.

In the case of the constant  $R$ , the values obtained for each of the four lots are approximately the same; the average value of  $R$  is very nearly 0.92.

### CONCLUSION

It seems, therefore, that, so far as the writers' results are concerned,  $M$  and  $A$  can be considered only as empirical constants and should not be thought of as representing, respectively, the maximum live weight *actually* to be attained and the *actual* gain necessary

<sup>7</sup> The most probable reason that these lots were not strictly comparable at the tenth week is that different amounts of feed had been consumed by the chicks in each of the pens. The reason for this difference in feed consumption is not clear. However, since some of the feed was fed according to the judgment of the attendant as to how much the birds would eat, and since in two of the pens the number of birds was smaller than in the other two pens, it is probable that more feed, per chick, was received by the birds in the pens containing fewer birds than in those containing the larger number. This suggestion as to the cause of the observed differences agrees with the facts.

## THE ACCURACY OF CATTLE WEIGHTS<sup>1</sup>

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### INTRODUCTION

It has been known for a long time that weights of cattle are subject to considerable fluctuation from day to day. Such fluctuations are of decided importance to research workers who are conducting experiments involving gains or losses in the weight of cattle. In a general way two classes of fluctuations may be distinguished: The first consists of sudden, rather large changes in the average weight of a group in which all, or nearly all, of the cattle gain (or lose) together, although not always to the same extent. Such changes may properly be called "fills" or "shrinks" in accordance with market usage of these terms, and the causes of such changes are usually readily recognizable in some environmental circumstances, such as a sudden drop in temperature, shipment to market, turning on pasture, etc. The second class consists of daily weight changes (either gains or losses) which individual animals may make, and which are independent of the daily trend of the average weight of their group. Both classes of weight fluctuations are encountered in feeding experiments. As an illustration of extreme and minimal "shrinks" and "fills" in weight among groups of experimental cattle may be cited the weights obtained on some dairy cows in a feeding experiment at College Station, Tex., in 1927. Twenty-four cows were weighed for three successive days in February, and 19 of the same cows, together with 10 others, were weighed for three successive days in April. The average weights were as follows:

	Feb. 16-18	Apr. 13-15
First day.....	856 pounds	824 pounds
Second day.....	849 pounds	822 pounds
Third day.....	813 pounds	821 pounds

The variation found in the February weights in this case was the most extreme in the writers' observation of dairy cows, yet in several other instances the range between the largest and smallest of these average weights for three successive days has exceeded 20 pounds. The weather records show clearly the cause of the heavy shrink from the second to the third weight in February, since in the 23 hours preceding the third day's weight the temperature dropped 52° F. and during part of that time a light mist fell. The cows were miserable in the freezing temperature, to which they were unaccustomed, and

<sup>1</sup> Received for publication Dec. 16, 1927; issued May, 1928.

<sup>2</sup> The statistical methods used in this study were selected and the calculations were made by the senior author. Indebtedness is hereby acknowledged to the following men for their part in collecting the original data and for helpful suggestions in interpreting these data: J. H. Shepperd, North Dakota Agricultural Experiment Station; F. E. Keating, of the Big Spring Station, Bureau of Plant Industry, U. S. Department of Agriculture; J. M. Jones, Fred Hale, R. E. Dickson, J. H. Jones, and W. E. Flint, of the Texas Agricultural Experiment Station; and E. W. Sheets, R. H. Tuckwiller, A. T. Semple, Bradford Knapp, jr., and E. W. McComas, of the Bureau of Animal Industry, U. S. Department of Agriculture.

naturally drank very little water. Possibly such variations may ordinarily be greater in mature dairy cows than in beef steers, but that large daily fluctuations do occasionally occur even among large groups of beef steers may be illustrated by the following average weights for a group of 72 North Dakota steers in the Mandan grazing trials for 1923. The small first weight is doubtless due to the fact that the steers were weighed directly from the cars after a shipment (from Mandan to Fargo) which caused them to be without feed and water for over 24 hours.

Date weighed:	Average weight in pounds	Date weighed:	Average weight in pounds
October 30.....	823	November 3.....	886
October 31.....	876	November 5.....	894
November 1.....	885	November 6.....	902
November 2.....	887	November 7.....	900

### THE USE OF CONTROL LOTS

Research workers generally have sought to eliminate the experimental errors arising from such group fluctuations in weight by using control or check lots and studying not the absolute gain or loss of the experimental lot in pounds but the difference between such gain or loss and the gain or loss made by the control lot which is weighed on the same day and is given as nearly as possible the same treatment as the experimental lot except for the one variable which is the object of study. Undoubtedly this procedure has eliminated much of the experimental error which would be involved in using the actual weights without comparison with the weights of a control lot. However, because individual animals do not vary in weight exactly as their group average does, not all the error is thus eliminated. For example, in the weights given above for the North Dakota steers, between November 6 and November 7 steer No. 85 gained 20 pounds, while steer No. 88 lost 15 pounds, steer No. 90 lost 10 pounds, and steer No. 91 gained 10 pounds. As long as such fluctuations independent of the daily trend of the group occur, there is always the possibility that the control lot will by "chance" contain a larger number of plus or of minus fluctuations than the experimental lot, and this may lead to more or less serious errors in interpreting the data.

This leads to the precise question which is the subject of study in this paper: How large are the experimental errors which can not thus be eliminated by the use of control lots in carefully conducted experiments, and are there any recognizable circumstances, such as the age, size or gentleness of the cattle, type of ration being fed, and weather conditions, which cause this experimental error to be larger or smaller than normal and which can be used in estimating the size of the error even before the weights are taken, if necessary.

### WEIGHING FOR THREE OR MORE DAYS

Most research workers have sought to eliminate this experimental error by weighing the cattle for three successive days and using the average of the three weights thus obtained as the correct weight instead of using a single day's weight. Doubtless the use of three-day average weights has helped to eliminate much of the experimental error caused by the "chance" fluctuations of individual

weights. Still, no one who has studied the subject would maintain that all of the experimental error has thus been eliminated. One need only weigh cattle for four successive days to find out that the fourth day's weights do not correspond exactly to the average weight for the first three days, nor does the four-day average correspond exactly to the three-day average. Armsby (*1*)<sup>3</sup> long ago pointed out that even a 10-day average weight did not entirely eliminate the influence of daily fluctuations in weight. Moreover, it sometimes happens that there are serious practical objections to weighing for three successive days. For example, it frequently happens in the case of grazing experiments that the scales are located at a considerable distance from some of the pastures, and the investigator may well wonder whether the unusual amount of excitement and exercise to which his cattle are subjected in being rounded up and driven in to the scales on the two additional days does not introduce errors greater than are eliminated by weighing for three days instead of one. It is therefore very desirable for the investigator to know just what increase in accuracy is gained by weighing for three days instead of one.

#### SCOPE OF THIS STUDY

The results of a preliminary study of this subject were presented at the annual meeting of the American Society of Animal Production at Chicago in November, 1926, and an abstract of the report there made appears in the proceedings of the meeting (*7*). The interest there expressed was largely responsible for a determination to enlarge the study to its present scope and to include weights taken under such widely different climatic conditions as prevail in two different regions in North Dakota (Mandan and Fargo), one in West Virginia (Greenbrier County), and four in Texas (Big Spring, College Station, Kingsville, and Spur). The data include weights of calves, yearlings, and 2-year-olds, thin and fat, in feed lots; yearlings, 2-year-olds, and 3-year-olds on pasture in the spring and in the fall, and dairy cows. Cattle of Hereford, Shorthorn, Aberdeen-Angus, Jersey, and Brahman breeding are included. Altogether, 9,897 individual weights were taken on more than 900 different animals. All animals were weighed at least on 3 consecutive days and some were weighed on as many as 11 consecutive days. Statistically, the total amount of data is equivalent to that which would be obtained by weighing 3,360 animals individually on 3 consecutive days, 672 animals on 11 consecutive days, or 1 animal, whose weight was not showing any consistent increase or decrease under absolutely uniform weather and other environmental conditions, on 6,721 consecutive days.

#### SPECIAL PECULIARITIES OF THE DATA STUDIED

The weights were not all taken with the same degree of precision. Thus most of the Kingsville weights and many of the Fargo weights and some of the Spur weights were taken to the nearest pound; the dairy weights, most of the Spur weights, and some of the Big Spring weights were taken to the nearest 2 pounds, while the West Virginia weights, Mandan weights, and most of the Big Spring weights were taken to the nearest 5 pounds.

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<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 579.

Some of the weights were taken on cattle which were rather wild and excitable (as the initial Kingsville weights), and other weights were taken on cattle which had become rather gentle (as the final weights in the feeding experiments), or on cattle which had been thoroughly gentle for a long time (as the dairy weights).

Some of the cattle were getting nothing to eat but pasture; others were getting both pasture and concentrates; many others were getting concentrates, dry roughage, and large amounts of silage; others were getting only concentrates and dry roughage and were confined at all times to the feed lot; and still others were getting dry roughage while on pasture.

Some of these peculiarities doubtless affect the accuracy of the individual weights, and these effects will be considered later.

#### STATISTICAL METHODS USED IN THE PRELIMINARY STUDY

In the preliminary report made at the 1926 meeting of the American Society of Animal Production, multiple correlation methods were used to calculate the standard error of estimating the average weight for an infinite number of days from a single day's weight or from the average of  $n$  days' weights. This calculation involved the assumption that the average correlation found between the weight of the same animals on the different days when they actually were weighed was the average correlation which would be found between weights of these same animals for any two different days if weighed for an infinite number of days. It also involved the assumption that the average of the three or more standard deviations actually found for the weights on the three or more days when weights were taken was the standard deviation of the weights of that particular group of cattle on any particular day. The error introduced by these assumptions was very slight, but was, nevertheless, undesirable. Since the printing of that report the senior author's attention was called to the fact that, by the use of intraclass correlations, difficulties in averaging correlations and standard deviations could be avoided, and thus both the above assumptions could be reduced to the assumption that the data actually studied were a random sample of other weights which might be taken on the same group of cattle, an assumption which is inherent in almost all statistical studies.

The method used in the preliminary report also differed in two other respects from the method used in the present study. In the first place, the ordinary biometrical formula for the standard deviation ( $\sqrt{\frac{\sum d^2}{n}}$ ) was used in the former, while the analysis of variance which was employed in the present study necessarily makes use of what is known as Bessel's formula ( $\sqrt{\frac{\sum d^2}{n-1}}$ ) or some modification of it to correspond to the number of "degrees of freedom" <sup>4</sup> in the data. In the second place, the standard errors given in the preliminary report were the standard errors of estimating the average of an infinite number of weights (as the dependent variable) from a single weight or from an average of two or more days' weights (as the

<sup>4</sup> See Fisher's *Statistical Methods for Research Workers*, p. 53 and 54, for discussion of which formula is most appropriate to use, and Chapters VII and VIII for further explanation of the idea of degrees of freedom (5).

independent variable). Now in actual practice investigators do not allow for the idea of regression in their estimate of the average of an infinite number of weights. Instead they say, "The average of the three days' weights was taken as the true weight," or some similar statement. The standard error of such estimates which ignore regression is the standard error of estimating (by the correct regression equation) a single weight or a two-day or three-day average weight (as the dependent variable) from the average of an infinite number of weights (as the independent variable). Both of these ideas can be incorporated in the multiple correlation method, and when that is done the results of that method and the "analysis of variance" method used in this study become identical, as of course they should if both methods are correct in principle.<sup>5</sup>

The difference between the figures as printed in the original report and as calculated in this study is very small and is almost entirely due to the use of Bessel's formula in this study instead of the usual biometrical formula which ignores the loss of degrees of freedom. Table 1 shows the standard errors as originally printed and as calculated by the analysis of variance.

TABLE 1.—*The experimental error in single weights of cattle as printed in the preliminary report and as calculated by the analysis of variance*

Experiment studied	Standard error of a single weight	
	As printed in the preliminary report	As calculated by the analysis of variance
	<i>Pounds</i>	<i>Pounds</i>
First Kingsville, initial.....	° 6.08	° 6.66
First Kingsville, final.....	° 6.35	7.41
First Big Spring, initial.....	° 7.06	7.29
First Big Spring, final.....	° 6.06	6.61
Second Big Spring, initial.....	5.67	5.83
Second Big Spring, final.....	° 7.94	8.56
Third Big Spring, initial.....	5.04	5.42
Third Big Spring, final.....	° 4.63	5.34

\* These figures were so calculated that the error is figured from the daily trend within each lot as a base instead of from the daily trend of the average weight of all lots combined. This has the effect of an assumption that differences in the daily trends of the weights of two lots of steers in the same experiment on the same weighing days are due to external differences in the ration or treatment and are not due to plus or minus fluctuations which are by chance greater in one lot than in the other. Either view might be correct according to the objects and conditions of each experiment.

<sup>b</sup> Through error in calculation this figure was printed as 7.49 in the preliminary report.

#### STATISTICAL METHODS USED IN THE PRESENT STUDY

Fisher's method (3) of analyzing variance (as explained in detail in Chapters VII and VIII of Statistical Methods for Research Workers) gives a "remaining variance" or "experimental error" which is identical with the standard error of estimating by multiple correlation methods a single day's weight from an average of an infinite number of weights, provided Bessel's formula is used for calculating the standard deviations. Fisher's method involves the idea of "degrees of freedom" which is involved in Bessel's formula for the standard deviation ( $\sqrt{\frac{\sum d^2}{n-1}}$ ), but is not involved in the for-

<sup>5</sup> The writers are especially indebted to Sewall Wright, of the University of Chicago, for his kindness in checking the statistical formulas used in this study.

mula ordinarily used by biometricians ( $\sqrt{\frac{\sum d^2}{n}}$ ). Statistical workers are rather generally agreed that the latter formula is the correct one where the standard deviation is to be applied to the sample from which it was calculated, as in calculating correlations or in fitting curves, but that the idea of "degrees of freedom" should be taken into consideration if the calculated standard deviation is to be regarded as an estimate of the true standard deviation existing in a very large population of which the actual data used in the calculation are only a random sample. The difference between the standard deviations as calculated by the two formulas is very small if  $n$  is at all large, being less than 4 per cent if  $n$  is as large as 12 and less than 1 per cent if  $n$  is as large as 50. Such difference as does exist causes the experimental error in cattle weights as figured by Fisher's method to be very slightly larger than the original figures given in the preliminary report for the errors in the one-day weights of cattle.

Briefly, the method of the analysis of variance which is used in this study consists of separating the total variance,  $\sum d^2$ , of all the individual weights into three portions: First, a portion due to the fact that the daily average weight is not identical on all three (or more) days of weighing; second, a portion due to the fact that the average weights of the different cattle for the whole period weighed are not identical; and finally, a remaining portion which is not due to either of these facts and which may properly be considered as experimental error. It is this remaining portion which is the object of study in this paper. Perhaps this remaining portion can be visualized better if we imagine a group of steers each of which is weighed for three (or more) days and all of which have identical average weights for the period of three days, and if we further imagine that weather and treatment were so uniform and equable during the days of the weighing that the average weights of the entire group for the different days were identical. Then in such a group of individual weights the standard deviation (with appropriate corrections for the number of degrees of freedom sacrificed in meeting the conditions imposed) would be the experimental error as found by the analysis of variance (or the standard error of estimating a single weight from the average of an infinite number of daily weights as found by the multiple correlation method). This amount of variation could be large or small, as illustrated by the hypothetical figures given in Table 2, in which the first two animals show a small degree of variation from day to day, while the last two show a large degree of variation.

TABLE 2.—Hypothetical weights showing day-to-day variation possible without variation in average weights

Steer No.	Hypothetical weights for—			Total
	First day	Second day	Third day	
	Pounds	Pounds	Pounds	Pounds
1.....	910	912	908	2,730
2.....	912	912	906	2,730
3.....	890	930	910	2,730
4.....	928	886	916	2,730
Total.....	3,640	3,640	3,640	10,920



To illustrate the method of calculation, in Table 3 are reproduced the actual data for the lot 3 West Virginia steers in 1925, and in Table 4 is given a summary of the analysis of the variance in the actual data presented in Table 3. The formulas and principles for obtaining the figures in Table 4 may be found in Fisher's book (3). Calculations similar to those in Table 4 were made for every group of cattle studied, and the standard deviations due to experimental error were then subjected to further study.

TABLE 3.—Weights of lot 3, West Virginia 3-year-old steers, 1925

Steer No.	Weight of steers on—			Total	Steer No.	Weight of steers on—			Total
	Apr. 21	Apr. 22	Apr. 23			Apr. 21	Apr. 22	Apr. 23	
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
3.....	920	925	910	2,755	51.....	960	965	975	2,900
6.....	830	820	815	2,465	54.....	940	975	960	2,875
9.....	775	770	770	2,315	57.....	1,050	1,040	1,040	3,130
12.....	1,090	1,105	1,085	3,280	60.....	1,010	1,015	1,000	3,025
15.....	1,015	1,000	1,000	3,015	61.....	890	875	895	2,660
18.....	850	860	855	2,565	66.....	945	945	950	2,840
19.....	870	850	845	2,565	69.....	885	880	870	2,635
24.....	905	905	905	2,715	72.....	930	920	910	2,760
27.....	845	845	845	2,535	75.....	960	965	955	2,880
30.....	960	950	940	2,850	78.....	1,035	1,040	1,050	3,125
33.....	1,065	1,050	1,065	3,180	81.....	1,070	1,070	1,060	3,200
36.....	1,010	1,020	1,025	3,055	84.....	880	875	870	2,625
39.....	965	975	970	2,910	87.....	1,080	1,060	1,055	3,195
42.....	1,085	1,075	1,075	3,235	90.....	1,020	1,010	1,010	3,040
45.....	960	960	935	2,855					
48.....	940	940	920	2,800	Total.....	28,740	28,685	28,560	85,985

TABLE 4.—Summary of the analysis of the variance in lot 3, West Virginia steers, for April, 1925

Variance due to—	Degrees of freedom	Sum of squares	Mean square	Standard deviation
All causes.....	89	617,911.388	6,942.82	83.32
Daily trend.....	2	567.222	283.61	3.07
Remainder.....	87	617,344.166	7,095.91	84.24
Difference between animals.....	29	613,461.388	21,153.84	83.97
Final remainder or experimental error.....	58	3,882.778	66.94	8.18

Table 4 is fairly representative of all such tables. In every case differences between animals were by far the most important causes of variation in the data. In nearly every instance the daily trend was a comparatively unimportant source of variation, although in a few cases it was important, and in the first Kingsville initial weights and in the February dairy cattle weights its standard deviation actually exceeded the experimental error.

### RESULTS OF THIS STUDY

A summary of the experimental error actually found in the different groups of cattle is presented in Table 5, along with other pertinent facts about the age, weights, ration, etc.

TABLE 5.—Average weight of cattle, together with precision of weights, standard deviation of average weights, degrees of freedom, and experimental error found in the different groups of weights

Group of weights studied	Number of cattle	Approximate age	Number of days weighed	Average weight (pounds)	Precision of weights (pounds)	Standard deviation of average weights	Degrees of freedom	Experimental error (pounds)	Notes *
First Kingsville, initial	100	9 to 11 months	3 and 4	520	1	77	216	6.7	A.
First Kingsville, final	67	15 to 17 months	3	745	5	73	132	7.4	B.
Third Kingsville, initial	90	16 to 22 months	3	628	5	82	196	11.9	C.
Third Kingsville, final	98	22 to 28 months	3	895	5	83	104	10.7	D.
First Big Spring, initial	42	13 to 15 months	3	717	5	69	143	6.6	C.
First Big Spring, final	45	18 to 10 months	3	506	5	45	88	5.8	D.
Second Big Spring, initial	45	14 to 16 months	3	809	5	68	88	8.6	E.
Second Big Spring, final	45	16 to 8 months	3	682	5	74	86	5.3	E.
Third Big Spring, initial	44	13 to 15 months	3	666	5	74	86	5.3	E.
Third Big Spring, final	22	32 months	3	840	2	80	42	14.6	F.
Spur, 1922-23	21	36 months	3	1,575	2	187	41	15.3	G.
2-year-olds, initial	25	24 months	3	876	2	94	48	6.2	G.
2-year-olds, final	25	32 months	3	769	2	96	46	11.2	H.
Yearlings, initial	24	20 months	3	1,131	2	131	38	14.9	H.
Yearlings, final	20	24 months	3	857	2	85	38	10.5	H.
Spur, 1925-26	24	21 months	3	632	2	74	56	6.2	L.
2-year-olds, initial	29	25 months	3	947	2	53	62	7.3	L.
Yearlings, final	32	9 months	3	422	2	46	62	6.1	L.
Calves, initial	31	13 months	3	686	2	68	60	7.2	L.
Calves, final	36	10 months	3	398	1	71	70	4.8	J.
Calves, initial	36	14 months	3	633	1	94	70	5.3	K.
Season, initial	30	14 months	3	552	1	87	58	10.5	K.
Season, final	30	18 months	3	736	1	106	58	8.9	K.
College station dairy cows	18	2 to 12 years	3	847	2	57	34	10.9	L.
In cottonseed-meal project—									
January, 1926	25	do	3	870	2	101	48	12.1	L.
January, 1927	24	do	3	886	2	102	46	10.1	L.
February, 1927	24	do	3	839	2	105	46	13.1	L.
March, 1927	34	do	3	804	2	105	66	16.0	L.
April, 1927	29	do	3	822	2	105	66	16.0	L.
May, 1927	34	do	3	860	2	112	54	13.6	L.
June, 1927	30	do	3	871	2	110	58	8.2	L.
July, 1927	26	do	3	848	2	108	50	12.1	L.

[illegible]

<sup>a</sup> The notes referred to in this column will be found immediately following the table.  
<sup>b</sup> The minus sign signifies that the degree of precision was slightly greater than the figure indicates. In this case most of the steers were weighed to the nearest 5 pounds, but a few were weighed to the nearest 2 pounds.  
<sup>c</sup> These steers were weighed to the nearest 5 pounds on the first day, but most of them were weighed to the nearest 2 pounds or less on the second and third days.

\* The notes referred to in this column will be found immediately following the table.

<sup>b</sup> The minus sign signifies that the degree of a few were weighed to the nearest 2 pounds.

a few were weighed to the nearest 2 pounds. These steers were weighed to the nearest 5 pounds on the first day, but most of them were weighed to the nearest 2 pounds or less on the second and third days.

TABLE 5.—Average weight of cattle, together with precision of weights, standard deviation of average weights, degrees of freedom, and experimental error found in the different groups of weights—Continued

Group of weights studied	Number of cattle	Approximate age	Number of days weighed	Average weight (pounds)	Precision of weights (pounds)	Standard deviation of average weights	Degrees of freedom	Experimental error (pounds)	Notes
North Dakota steers—Continued.									
1922-23 sweet-clover hay and corn silage, steers—									
Nov. 27-29	24	2½ years	3	961	5	111	46	8.5	Y.
Dec. 27-29	24	do	3	1,023	5	120	40	6.9	
Jan. 24-26	24	do	3	1,088	5	126	40	7.8	
Feb. 16-17	24	3 years	3	1,118	5	130	46	8.6	
Silage experiments, 1921-22—									
Dec. 8-9	20	2½ years	3	904	1	75	38	10.4	Z.
Jan. 7-9	20	do	3	1,015	1	87	38	7.3	
Feb. 6-8	20	do	3	1,036	1	94	38	8.6	
Mar. 8-10	19	do	3	1,081	1	92	36	9.4	
Mar. 18-20	19	3 years	3	1,088	1	89	36	10.1	
Silage experiments, 1922-23—									
Nov. 21-Dec. 1	19	2½ years	11	907	1	31	180	12.4	A.A.
Dec. 9-11	19	do	3	956	1	32	36	12.0	
Dec. 20-31	19	do	3	975	1	54	36	12.5	
Jan. 29-31	19	do	3	1,008	1	62	36	8.8	
Feb. 27-Mar. 1	19	do	3	1,026	1	70	36	7.9	
Mar. 29-Apr. 1	19	3 years	4	1,099	1	81	54	9.5	
Silage experiments, 1923-24—									
Dec. 15-22	20	2½ years	8	892	1	50	133	10.1	A.B.
Dec. 30-Jan. 1	20	do	3	918	1	51	38	15.1	
Jan. 10-21	20	do	3	957	1	58	38	8.9	
Feb. 18-20	20	do	3	1,002	1	69	38	12.6	
Mar. 19-21	20	do	3	1,057	1	82	38	9.4	
Apr. 18-20	20	do	3	1,112	1	95	38	7.7	
Apr. 27-29	20	3 years	3	1,150	1	99	38	9.1	
Silage experiments, 1924-25—									
Nov. 11-21	20	2½ years	11	960	1	48	100	13.9	A.C.
Dec. 19-21	20	do	3	1,055	1	53	38	10.9	
Jan. 17-19	20	do	3	1,107	1	59	38	16.5	
Feb. 16-18	20	do	3	1,160	1	76	38	14.5	
Mar. 19-21	20	do	3	1,209	1	96	38	7.7	
Apr. 8-10	20	3 years	3	1,230	1	102	38	8.9	
Silage experiments, 1925-26—									
Nov. 22-27	20	2½ years	6	930	1	63	95	11.8	A.D.
Dec. 6-7	20	do	3	958	1	65	38	9.1	
Dec. 25-27	20	do	3	995	1	65	38	11.7	
Jan. 24-26	20	do	3	1,045	1	75	38	7.9	
Feb. 23-25	20	do	3	1,104	1	82	38	11.6	
Mar. 26-27	20	do	3	1,168	1	94	38	5.5	
Apr. 14-16	20	3 years	3	1,200	1	95	38	9.2	

## NOTES

A. These calves had just been separated from their dams on the range. One lot consisted of Herefords, another of Shorthorns, another of crossbred calves out of Hereford cows but by high-grade Brahman bulls; the fourth lot consisted of crossbred calves out of Shorthorn cows but sired by high-grade Brahman bulls. They were fed for 179 days in dry lots on a fattening ration of corn or grain sorghum, cottonseed meal, Rhodes grass hay, and a little sorghum silage.

B. These steers had been grazing on fairly good pasture but without grain for several months before the feeding began. Two lots consisted of Hereford and Shorthorn steers, the third lot consisted of crossbred steers out of Shorthorn cows but sired by high-grade Brahman bulls, and the fourth lot consisted of steers out of cows and by bulls identical in breeding with the third lot of steers. They were fed in dry lots for 175 days on fattening rations of ground grain sorghums or ground corn, cottonseed meal, and Rhodes grass hay or hegari stover.

C. These calves were Herefords which had been weaned about two weeks when the experiment began and had been on preliminary feed in dry lots during that time. They were fed in dry lots for 175 days on fattening rations of ground milo heads, cottonseed meal, and sorgo fodder or cottonseed hulls or sorgo silage and Sudan grass hay. Described in more detail in Bulletin No. 363 of the Texas station (4).

D. Same as C, except that the calves had been weaned, dehorned, and branded just three days before the experiment began and the fattening period was only 168 days.

E. Same as C, except that the calves had been weaned only three days before the experiment began and the fattening period was 203 days in length.

F. These steers were Herefords which had been on rather poor pasture all summer and were quite thin. One lot was fed cottonseed meal and hulls. The other lot received ground shelled corn in addition. They were full fed for 120 days in a dry lot. Described in detail in Bulletin 309 of the Texas station (5).

G. These steers had been on rather poor pasture all summer and were quite thin. One lot consisted of Herefords and the other lot consisted of crossbred steers out of Hereford dams but sired by high-grade Brahman bulls. They were full fed for 120 days in a dry lot on a ration of ground shelled corn, cottonseed meal, and hulls.

H. These steers had been on good pasture until late in the summer and had been shipped to Spur about two weeks before the initial weights were taken. Half of them of each age were Herefords and the rest were crossbred Hereford-Brahmans, like those described in G. They were full fed for 112 days on a ration of ground milo heads, cottonseed meal, cottonseed hulls, and sorghum hay.

I. These steers had been on good pasture until the middle of October, when they were shipped to Spur. They were fed a moderate grain and sorghum fodder ration in dry lots at Spur for a month before the first weights were taken. There were three lots of each age. One lot consisted of Herefords and one lot of Hereford-Brahman crossbreds. The third lot was sired by a Hereford bull out of Hereford-Brahman crossbred cows. They were full fed in a dry lot for 112 days on a fattening ration of ground milo heads, cottonseed meal, and chopped sorghum fodder.

J. These calves were of the same three kinds of breeding as those described in I. They had been on fairly good pasture until weaned in the middle of October and shipped to Spur. They were kept on a moderate grain and sorghum fodder ration in dry lots for about two months before the initial weights were taken. They were full fed in dry lots for 111 days on a fattening ration of ground milo heads, cottonseed meal, and chopped sorghum fodder.

K. These steers were long-aged calves from the S M S ranch. They were predominantly of Hereford breeding, but several showed very strong traces of Shorthorn blood. They had been driven from near-by pastures and put in the feed lots at Spur on a moderate grain and sorghum fodder ration more than a month before the first weights were taken. Some of them already carried considerable fat. They were full fed in dry lots for 111 days on a fattening ration of ground milo heads, cottonseed meal, and chopped sorghum fodder.

L. These were high-grade or purebred Jersey cows which were being used in feeding experiments. The first experiment is described in Bulletin 352 of the Texas station (8). The cottonseed-meal experiments are not yet published. The cows received concentrates in proportion to milk production, a small amount of sorghum silage, an unrestricted quantity of cottonseed hulls, and pasture (either native or oat) whenever it was available. All cows in the experiment

station herd were used in these tests if in suitable stages of lactation and not on register of merit tests.

M. These calves were driven not to exceed one-half mile to the scales. They were on pasture, but were also given hay or corn stover after the first and second day's weighings. They were good to choice Hereford steer calves, obtained in north-central Texas. The cattle and rations are described in detail in United States Department of Agriculture, Department Bulletin No. 1431 (10) and in West Virginia Agricultural Experiment Station Bulletin No. 218 (12).

N. Driven not to exceed 200 to 300 yards to the scales in the morning after being fed but before being watered. Had been fed wintering rations in lots in an open barn. Same cattle as in M.

O. Same cattle and treatment as in M, except that cattle were a year older.

P. Same cattle and treatment as in N, except that cattle were a year older and lot 3 had received corn silage and wheat straw instead of the grain mixture received by lot 3 the previous winter.

Q. Same cattle and treatment as in M, except that the cattle were now 2 years older and also that after the first day's weighing they were moved a distance of 6 miles. It seems likely that this drive was in large part responsible for the uniformly high experimental errors found at this weighing.

R. Same cattle and treatment as in N, except that the cattle were 2 years older and all had been wintered on the same rations, that is, cottonseed meal, corn silage, and wheat straw.

S. These were steers of all three leading beef breeds. They had been shipped from Mandan to West Fargo and the first day's weights were taken soon after they were unloaded and driven 4 miles to the college cattle yards. They had received neither hay nor water for more than 24 hours before the first weighing. After the first weighing they were grazed on cornfield gleanings and meadow aftermath and were also given some hay.

T. These were steers of all three leading beef breeds. They were on prairie pasture and had been in the pasture where weighed for two days before the first weights were taken and on various near-by pastures for two to three weeks before weighing.

U. These were steers of all three leading beef breeds. They were weighed the first day direct from the stock cars without having had feed or water. They had been shipped from Mandan, where they had been on prairie pasture, to West Fargo and had been in transit more than 24 hours. After the first weighing they were allowed to graze on cornstalk field gleanings and meadow aftermath and were given some hay.

V. These six head were the same as those in U, except that they were not weighed on the first day.

W. Four of these steers were 2-year-old Herefords which had been used in digestion trials; two were 3-year-old Shorthorns in high stocker condition. They were turned out on sweet-clover pasture May 11. Three of them were changed to a light ration of alfalfa hay July 27 and continued on that through September. The four Herefords had been on a light ration before being turned out on the sweet-clover pasture on May 11, and therefore began to make good gains on pasture in about a week or 10 days. The two Shorthorns were turned on pasture directly from a fairly good grain ration and were already carrying a considerable amount of fat. Therefore they lost much weight at first. One of them became adjusted to the change in feed in a few days and regained his loss within the first month. The other continued to lose weight for nearly a week and did not regain all his loss for more than two months. Because of this difference in trends, both steers are omitted from the calculations for May 12-18, as explained in the discussion on pages 572 and 573. These steers are described in more detail in North Dakota Agricultural Experiment Station Bulletin No. 211 (11).

X. These steers were of all three leading beef breeds. They had been on cornstalk pasture and had received some hay for about a month just prior to the first day of weighing. They were fed a heavy ration of alfalfa hay and corn silage.

Y. Same as X, except that sweet-clover hay was used instead of alfalfa.

Z. These were steers of all three leading beef breeds. There were four lots, each receiving a different silage in addition to oat straw, cottonseed meal, and corn meal. Silages used were corn, sunflower, millet, and a mixed silage of sweet clover and oat straw.

AA. Same as Z, except that ground barley was fed instead of corn meal and an unmixed sweet-clover silage was fed to one lot instead of the millet silage used the preceding year.

AB. These were steers of all three leading beef breeds. There were four lots, each receiving a different silage in addition to alfalfa, linseed meal, and ground barley. The silages were corn, sunflower, a late cutting of sweet clover, and a mixture of equal parts of an early cutting of sweet clover and an early cutting of corn.

AC. Same as AB, except that corn silage was fed to two lots and sweet-clover silage to two lots.

AD. Same as AB, except that corn silage was fed to one lot, sweet-clover silage to two lots, and salted sweet-clover silage to one lot.

## DISCUSSION OF RESULTS

### SIZE OF EXPERIMENTAL ERROR

The outstanding conclusion to be drawn from Table 5 is that the experimental error is quite irregular, ranging from as low as 4 pounds to as high as 17 pounds. The average experimental error (regarding each group of animals as equal in importance to every other group, instead of the more laborious but more accurate method of squaring each error, multiplying it by the number of degrees of freedom on which it was based, adding, dividing the sum of these weighted squared errors by the total number of degrees of freedom, and extracting the square root of the quotient) is 10.02 pounds. The frequency distribution of the error is shown in Table 6.

TABLE 6.—*Frequency distribution of different sizes of experimental error*

Size of error (pounds)	Number of groups of cattle	Size of error (pounds)	Number of groups of cattle	Size of error (pounds)	Number of groups of cattle
4.0-4.9	1	9.0-9.9	11	14.0-14.9	4
5.0-5.9	6	10.0-10.9	8	15.0-15.9	2
6.0-6.9	9	11.0-11.9	11	16.0-16.9	5
7.0-7.9	13	12.0-12.9	7	17.0-17.9	2
8.0-8.9	18	13.0-13.9	6		

### MECHANICAL ACCURACY OF SCALES

Care was exercised at all times to see that the scales on which the cattle were weighed were in good mechanical condition. Precautions were taken to see that all bearings and knife edges were free from dirt and that there was no binding of the platform against the frame. A load was weighed in different positions on the scale platform to check the accuracy of the scale. In the 1926-27 experiment at Spur an automatic dial scale of modern design was installed especially for the purpose of assuring the highest possible accuracy in the weights of the cattle, and was tested by using twenty 50-pound standard test weights. Parenthetically, it may not be amiss to observe that while the calves at this weighing showed a very small experimental error, the older group of steers showed an experimental error of 10.5 pounds, which is of about average size.

In only one case was the scale suspected of being not entirely accurate and sensitive to the degree of precision with which the weights were taken. That was the scale on which the dairy cows were weighed. This scale was a wagon scale designed for much larger weights and its knife edges were not in the best condition at

all times. The dairy cows weighed on it showed a larger experimental error than did any other group included in this study, but they were also older and had larger digestive tracts, and it is not at all unlikely that these two latter circumstances were more responsible for the large experimental error than was any mechanical defect in the scale.

Moreover, the method of calculation was such that any error which was constant for an entire day would have been eliminated from the figures. For example, if the weights on any scale had been consistently 10 pounds lighter or heavier than they should have been on any one day, that error would have been eliminated with the daily trend, and hence would not affect the size of the experimental error, which is the subject of this study.

In view of all these facts, it seems safe to conclude that mechanical inaccuracy in the use of the scales had little or no effect upon the size of the experimental errors studied here. However, it might be well to point out that such errors as may possibly have been due in these data to mechanical inaccuracies of weighing will be encountered in other experimental work with cattle unless the workers take even greater precautions than were taken in these experiments to insure accuracy of weights. Therefore the general applicability of the conclusions of this study can hardly be questioned on the grounds of mechanical inaccuracy of the scales, unless it is maintained that the precautions listed above are totally inadequate to insure an accurate weight.

#### AGE OF CATTLE

In an effort to analyze the causes of the variation in the size of the experimental error, several variables were studied. Age seems to have an important bearing on it, as is shown in Table 7.

TABLE 7.—Average experimental error for cattle of different ages

Age of cattle	Number of groups	Unweighted average error (pounds)
1 year and under.....	12	6.6
2 years and over 1 year.....	22	9.0
Over 2 years.....	69	11.0

However, the correlation between age and size of error is by no means close. Thus many of the 2 and 3 year old steers have errors as low as 8 pounds and a few have errors less than 6 pounds. It is safe to conclude, however, that as a general rule the size of the experimental error to be expected increases somewhat with the age of the animals.

#### WEIGHT OF CATTLE

Weight also seems to have an influence on the size of the error, as shown in Table 8, but this is irregular, and an inspection of the detailed results as given in Table 5 indicates that weight is related to the size of the error only in that average weight and the size of the error are both partially determined by age.



TABLE 8.—*Unweighted average experimental error for cattle of different average weights*

Weight of cattle (pounds)	Number of groups	Average error (pounds)	Weight of cattle (pounds)	Number of groups	Average error (pounds)
300- 399	5	6.4	800- 899	20	12.1
400- 499	5	6.8	900- 999	20	11.5
500- 599	8	9.0	1,000-1,099	16	9.0
600- 699	9	8.5	1,100-1,199	13	10.9
700- 799	4	8.6	1,200-1,299	3	8.6

That it is not weight which influences directly the size of the error is probably shown best by the steers which were fattened in dry lots, as the 13 groups of Kingsville, Big Spring, and Spur steers, which increased their weights by 40 to 115 per cent within four to six months, but showed an average experimental error of 8.8 pounds in their initial weights and an average experimental error of 8.5 pounds in their final weights.

The same thing is shown by the North Dakota silage experiments with fattening steers in dry lots where three-day weights were taken at intervals of 30 days or less. The average errors for the first three-day weights, second three-day weights, etc., are shown in Table 9.

TABLE 9.—*Average error at different periods of fattening, North Dakota silage experiments*

Order of weights	Number of groups averaged	Average error (pounds)	Order of weights	Number of groups averaged	Average error (pounds)
Initial.....	7	10.9	Fifth.....	5	9.3
Second.....	7	9.9	Sixth.....	4	7.9
Third.....	7	10.4	Seventh.....	2	9.2
Fourth.....	7	10.2			

Here it is clear that there is no tendency for the size of the error to increase with increasing weight. In fact, a straight line fitted to these data shows an average decrease of 0.36 pound in the error from one period to the next. This decrease is on the border line of significance statistically, there being about one chance in thirty that such a decrease could have happened merely as a matter of chance. It seems reasonably certain that there was a real but slight tendency for the error to decrease as the feeding period progressed, in spite of the fact that the average weight of the cattle was increasing rapidly. However, weight was not the only variable which changed with the progress of the feeding period. Doubtless the steers became gentler and less excitable at weighing time as the feeding period progressed, although of that there is no quantitative measure. Possibly a more important variable is the changing nature of the ration, which consisted largely of silage at the beginning, but which was made up more and more largely of concentrates as the feeding progressed. At all events, weight by itself is not a very important factor in determining the size of the experimental error. Such importance as it does seem to show in Table 8 may more logically be attributed to age and to the size of the digestive organs. Quite

possibly there might be found a much closer relation between size of the error and the fat-free live weight instead of the gross live weight, but it seems clear that increasing fatness has no tendency to increase the size of the error.

#### NATURE OF THE RATION

As mentioned in the preceding section, the increase in the proportion of concentrates in the ration during fattening may possibly be the cause of the slight decrease in the error observed during the progress of the feeding period. It is worthy of note that the errors of many of the groups of animals on pasture were rather large. In contrast to this, however, are such small errors as those of the Mandan steers for May, 1923, and the 1923 fall weights of the West Virginia yearlings which were also grazing on pasture. Moreover, most of the cattle grazing on pasture were older than those in feed lots. The experimental errors given in Table 5 are so irregular with respect to the rations which the different lots received that no clear-cut effect of ration upon the size of the error is evident.

Some of the data have a definite bearing on this point. For example, the Spur 1922-23 2-year-olds were divided into two lots, one of which was fed cottonseed meal and hulls, while the other was fed ground shelled corn, cottonseed meal, and hulls. (The steers and the rations are described in detail in Bulletin No. 309 of the Texas station (5).) The rations are distinctly different, especially as to proportion of concentrates included, yet the difference in the size of the experimental error is insignificant, being barely larger than its standard deviation.<sup>6</sup> In the Spur 1926-27 final weights for the Swenson steers there were three lots of steers all receiving milo heads, sorgo fodder, and cottonseed meal. In one lot the sorgo fodder was run through an ensilage cutter and chopped finely before feeding. In another lot the milo heads were fed unground. The lot receiving chopped fodder and ground heads was the most erratic in weight; the lot receiving whole heads and unchopped fodder was least erratic. However, two of the three differences are certainly not significant statistically, and the largest difference is only 2.6 times its standard deviation, and therefore its significance is still open to reasonable doubt.

The final weights of the Big Spring steers also have a bearing on this point when analyzed separately for each lot. (Steers and rations are described in detail in Bulletin No. 363 of the Texas station (4) and in Technical Bulletin No. 43 of the United States Department of Agriculture (2).) The weights of the steers receiving cottonseed hulls as a roughage were the least erratic in two of the three trials. The weights of the steers receiving sorgo fodder were the most erratic in all three trials. The weights of the steers receiving sorgo silage were least erratic in one trial and intermediate in the other two. However, when the differences in the size of the experimental error are tested for statistical significance, only the difference between the lots fed hulls and those fed sorgo fodder approach significance. That difference is 2.6 times its standard deviation, which makes the

<sup>6</sup> The criterion used in this section for determining significance or nonsignificance of differences in the size of the experimental error is the *Z* test described in Fisher's *Statistical Methods for Research Workers* (5), p 192-200.

probability that such a difference could have happened by chance slightly less than 0.01.

In the third Kingsville experiment two rations were fed. Each was fed to a lot of Hereford and Shorthorn steers and also to a lot of steers of mixed Shorthorn and Brahman breeding. The rations differed only in the roughage, which was Rhodes-grass hay in one and hegari stover in the other. In the initial weights of the Brahman, those on the Rhodes-grass hay gave the smaller error (see Table 11); of the non-Brahmans the more erratic weights were obtained from those on Rhodes-grass hay. As the differences barely exceed twice their standard deviation, it is concluded that they are not significant. In the final weights the more erratic are given by the groups getting hegari stover in both cases, the average difference being more than four times its standard deviation, thereby indicating a significant difference. This fact of course does not eliminate the possibility that this real difference may have been due to some other common cause, such as the order in which the lots were weighed rather than to the difference in the nature of the ration. Physically the two rations were not very different, as the hay and the stover were both dry feeds and were chopped into short lengths.

The three lots of West Virginia yearlings in the spring of 1923 show no significant difference in the size of their experimental errors, although their rations during the preceding four months were quite different, two of them including silage and straw, with either clover hay or cottonseed meal, while the other lot received mixed hay and a grain mixture. (A detailed description of the cattle and of the rations used will be found in United States Department of Agriculture Bulletin No. 1431 (10).) The West Virginia steers during the winter of 1923-24 all received corn silage and wheat straw. In addition, one lot received clover hay, one received cottonseed meal, and one received mixed hay. Yet the experimental errors found at the end of 120 days in two of the lots on these rations were identical, and the difference between those two and the third barely exceeded its standard deviation.

The weights of the North Dakota steers were not subjected to analysis on this point because the groups on the different rations were small (only five steers in most of them), and the rations in most cases differed only in the kind of silage used, which was usually corn or sweet clover, but also included millet and sunflower silages in some cases.

In surveying this whole group of data on whether or not the nature of the ration affects the size of the experimental error, one is impressed by the fact that nearly all the data show no effect or are contradictory in cases in which the experiment was repeated. Only in the Kingsville data do the figures compel the belief that anything other than chance is needed to account for the observed differences, and even in that case the significance arises almost entirely from the very large error in only one of the lots receiving hegari stover, the last lot weighed; so that it is easy to infer that some special circumstance operated to make the weights of lot 4 significantly more erratic than those of the Brahman lot receiving the same ration or those of the other two lots receiving Rhodes-grass hay. The magnitude of the difference between the fodder-fed lots and the hull-fed lots at Big Spring and

the fact that the difference is in the same direction in all three tests is very suggestive of a real difference. Also the magnitude of the difference between the lot fed chopped fodder and ground heads and the lot fed bundles and whole heads at Spur in 1926-27 is suggestive, although it is based upon only a single test. The writers' conclusion from these data is that the nature of the ration has little influence upon the size of the experimental error, if, indeed, it has any at all.

It should be remembered that all the rations represented in these data were reasonably palatable and not very likely to produce scouring or to cause the cattle to go off feed in any other way. Nothing in these data should be construed as proving that an unpalatable ration, or one which can easily cause scouring and other digestive disturbances in some of the cattle may not cause the weights to be more erratic than weights of similar cattle on a standard ration.

#### PRECISION OF WEIGHTS

The precision with which the weights were taken seems to have little influence upon the size of the error, as shown in Table 10.

TABLE 10.—*Unweighted average experimental error for cattle weighed with different degrees of precision*

Precision of weights	Number of groups	Average error (pounds)
1 pound.....	41	9.9
2 pounds.....	22	10.7
5 pounds.....	40	9.8

However, the size of the experimental errors found does tell something of the degree of accuracy that is sacrificed by weighing cattle only to the nearest 5 pounds instead of to the nearest 2 pounds or the nearest pound. Fisher (3) makes the following statement on this point: "It has been shown that as regards obtaining estimates of the parameters of a normal population, the loss of information caused by grouping is less than 1 per cent, provided the group interval does not exceed one quarter of the standard deviation." Weighing cattle to the nearest 5 pounds is equivalent to weighing them to a much finer degree of precision and then grouping the weights in 5-pound intervals for calculation. What is called "experimental error" in this paper is the standard deviation with which the grouping interval should be compared to determine whether or not the grouping is too coarse. Upon making this comparison it is found that a 1-pound interval is less than one-fourth of the experimental error in every one of the 103 groups of cattle, and therefore that practically nothing would be gained by weighing cattle of these kinds to a still finer degree of precision, e. g., to half pounds. A 2-pound interval is found to be more than one-fourth of the experimental error in 29 of the 103 groups of cattle. Therefore, there is a loss of slightly more than 1 per cent of the information by the use of 2-pound intervals in some of the groups, particularly in the younger cattle kept under the most uniform conditions. However, for most of the cattle a 2-pound interval involves practically no loss of information. On the other hand, the 5-pound

interval is more than one-fourth of the experimental error in all of the groups of cattle, and therefore involves a loss of considerably more than 1 per cent of the information. On the basis of these findings it would seem that cattle should be weighed at least to the nearest 2-pound unit in experimental work, and that if they are young and are being kept under quite uniform conditions, weighing to the nearest pound would be still more accurate. Weighing to units smaller than 1 pound would not result in any additional accuracy in cattle of the ages included in this study, although it might perhaps be advisable in studies of birth weights and other weights of calves up to weaning time.

Weights of groups of steers of course have experimental errors larger, in proportion to the square root of the number of steers in the group, than weights of single steers. Thus a commercial stockyard scale weighing a group of 25 steers in 10-pound units is no less precise than an experimental scale weighing the same steers one at a time in 2-pound units.

In this discussion of precision it has been assumed that the scales used are kept as accurate as their construction will permit and are quite sensitive, at least to the degree of precision used. Now, as a matter of fact, scales are not usually kept in as good condition as that. Dust and dirt get in the bearings, dulling their sensitiveness somewhat; a strong breeze blowing against the scale rack may cause the beam to vary its reading slightly from time to time; the weigher may be in so much of a hurry that he does not always bring the beam to exactly the same position each time before reading the weight. These and other circumstances keep the scales from being exactly accurate, even though research workers generally take every practical precaution to safeguard the accuracy of their scales and of the weights which they obtain. This makes it still more important to weigh experimental cattle with a fine degree of precision. Thus, if the scale breaks slowly, an impatient weigher weighing only to 5-pound units might easily read the weight 5 pounds too high or 5 pounds too low, whereas if he were weighing to 2-pound units he would read it only 2 pounds too high or 2 pounds too low. For this additional reason the writers believe that accuracy is gained by weighing experimental cattle at least to the nearest 2 pounds, and, if they are quite small, to the nearest pound.

The dairy cows used in these experiments were weighed upon a set of old wagon scales which had never been very sensitive, even when new, and the edges had become dulled, so that it was stiff and slow in breaking. This may have been in part responsible for the fact that the weights of these cows showed higher experimental errors than the average of weights taken elsewhere.

#### BREED AND GENTLENESS OF CATTLE

Complete comparisons between different breeds of cattle are not possible, as usually only one or two breeds were weighed in the same experiment. However, it may not be amiss to point out that the Jersey cows (which were gentle) showed a rather large error, although the size of their digestive organs and perhaps the condition of the scales on which they were weighed probably influenced the size of the error more than any breed characteristics.

The Kingsville weights and all the Spur weights, except the 1922-23 2-year-olds and the 1926-27 Swenson steers, furnish as good evidence as could be desired of the influence (if any) of the gentleness or wildness of the cattle on the size of the experimental error. In all these experiments some of the lots consisted of steers carrying Brahman blood in amounts ranging from three-sixteenths to as much as seven-sixteenths. Other lots weighed on the same days and under the same conditions consisted entirely of high-grade Herefords or Shorthorns. On an average, the Brahman steers were much wilder and more excitable than the other steers, although there was much variation in the disposition of different Brahman steers. Most of the Brahmans improved in gentleness during the fattening period more than the non-Brahmans, but the slight unusual commotion on weighing days was enough to arouse most of the Brahmans to a high pitch of excitement even at the close of the feeding period.

To determine whether the errors actually were greater in the case of the Brahmans, the error was calculated for each lot of steers separately instead of using as one lot all the steers weighed on the same day as was done in the main part of this study. The results of this more detailed treatment of the Kingsville data are given in Table 11.

TABLE 11.—*Experimental error in separate lots, Kingsville data*

Lot numbers in Kingsville weighings	Breed	Experimental error (pounds)	Degrees of freedom
First Kingsville, initial:			
1.....	Hereford.....	5.23	72
2.....	Shorthorn-Brahman.....	7.36	48
3.....	Hereford-Brahman.....	7.25	48
4.....	Shorthorn.....	5.50	48
First Kingsville, final:			
1.....	Hereford.....	8.39	32
2.....	Shorthorn-Brahman.....	4.93	32
3.....	Hereford-Brahman.....	5.90	30
4.....	Shorthorn.....	7.14	32
Third Kingsville, initial:			
1.....	Hereford and Shorthorn.....	5.13	48
2.....	Shorthorn-Brahman.....	4.59	48
3.....	do.....	5.29	46
4.....	Hereford and Shorthorn.....	4.50	48
Third Kingsville, final:			
1.....	Hereford and Shorthorn.....	8.31	46
2.....	Shorthorn-Brahman.....	6.42	46
3.....	do.....	7.93	48
4.....	Hereford and Shorthorn.....	14.89	48

When Fisher's *Z* test of the significance of differences in variance is applied to the figures in Table 11 it is found that in the first Kingsville initial weights the Brahmans were more erratic by a difference which might be expected to occur by chance only about once in four hundred times. On the other hand, in the first Kingsville final weights the Brahmans were the least erratic by a difference which might be expected to occur merely by chance even less frequently than that. In the third Kingsville initial weights the differences are small and probably not significant, although the differences between lots 1 and 3 on the one hand and lots 2 and 4 on the other approach statistical significance. However, those differences do not parallel either breed

ible explanation for them. In the third Kingsville final weights lot 4 is quite significantly more erratic than the other three lots, but the other differences are probably insignificant, the value of *Z* being less than twice its standard deviation. Thus we see that the Kingsville data are contradictory as to the existence of a breed difference. The fact that the digestive organs of the Herefords and Shorthorns were larger than those of the Brahman (as revealed by unpublished data obtained at slaughtering time) may account for the observed differences at the time of final weights. The large error for the final weights of lot 4 in the third experiments may be due to the fact that the final weights for this lot were taken at a much earlier hour the third day than on the other two days, so as to permit the cattle to be driven to the loading pens in the afternoon. No very plausible explanation for the higher errors of the Brahman in the first Kingsville initial weights has been advanced, unless the greater wildness of the Brahman may be invoked as such.

The Spur data also were recalculated to find the experimental error for each lot separately. The differences in the average experimental errors are shown in Table 12. The cattle called "Herefords" were high-grade Herefords, having probably six or seven top crosses of Hereford blood. Those called "Brahman" were out of the same kind of dams as the "Herefords," but were sired by a three-quarters or by a sixty-one sixty-fourths Brahman bull. They therefore carried a little less than one-half Brahman blood. Those called "back cross" were sired by a Hereford bull out of cows which were half or full sisters to the "Brahman" steers, and therefore the back-cross steers carried a little less than one-fourth of Brahman blood.

TABLE 12.—*Differences in experimental error found for steers of different breeding, Spur data*

Group of weights	Difference in experimental error (pounds)		
	Brahman minus Here- ford	Brahman minus back cross	Back cross minus Hereford
1922-23 yearlings, initial.....	+0.70		
1922-23 yearlings, final.....	+.02		
1924-25 2-year-olds, initial.....	-5.03		
1924-25 2-year-olds, final.....	-5.85		
1924-25 yearlings, initial.....	+2.30		
1924-25 yearlings, final.....	+.12		
1925-26 yearlings, initial.....	-.34	-0.51	+0.17
1925-26 yearlings, final.....	+1.02	-1.50	+2.52
1925-26 calves, initial.....	-.99	3.51	+2.52
1925-26 calves, final.....	+3.71	+1.19	+2.52
1926-27 calves, initial.....	-.22	+1.48	-1.70
1926-27 calves, final.....	+.13	-.61	+.74
Sum.....	-4.43	-3.46	+6.77
Average.....	-.37	-.58	+1.13
Probability.....	{ Between 0.6 and 0.7	Between 0.4 and 0.5	Between 0.1 and 0.2

The probability expressed in Table 12 is the probability that the observed average difference was not a real one—i. e., that it differed from zero only by chance. This is the test commonly known as "Student's method" of testing the significance of an average difference. From the high probabilities which were found it is obvious

that no one of the three kinds of steers was significantly more or less erratic in weights than either of the other kinds. Detailed examination of the data does show, however, that for some reason (not now apparent) the 2-year-old Herefords fed in 1924-25 were more erratic in their weights, both initial and final, than were the corresponding Brahmans. The probability of the observed difference being due to chance alone is less than 0.05 (*Z* test) in either initial or final weights alone and much less than that when both are considered together.

The data seem to justify the conclusion that wildness of the cattle or breed differences have little or no consistent effect upon the experimental error of the cattle weights within a lot. It should be pointed out, however, that wildness of the cattle may affect the error of a difference between two lots if one lot contains more nervous cattle than the other. For example, in the initial weights of the 1924-25 yearlings at Spur there was a distinct breed difference in daily trend. The Herefords were gentle, and their second day's weight averaged 5 pounds more than their first, while the third day's weight averaged 12 pounds more than the second. On the other hand, this particular group of Brahmans was the wildest that the writers have handled, and their second day's weight averaged 26 pounds less than their first, and their third day's weight averaged 8 pounds more than their second. Thus the daily trends were different in the two groups, and treating them as one group does not allow for this difference. When they are treated as two groups the differences in daily trend may be subtracted, and the experimental error is found to be 11.4 pounds, with 36 degrees of freedom, instead of 14.9 pounds, with 38 degrees of freedom, as it was when this breed difference in gentleness was not taken into account. This was the most extreme case found of a breed difference in daily trend.

It is believed that the data justify the conclusion that breed makes little difference in the accuracy of the weights, except in so far as it may be associated with differences in the size and capacity of the digestive tract or with differences in nervousness or excitability which are great enough to make the daily trends distinctly different in the lots compared.

#### UNIFORMITY OF CONDITIONS

Two groups of steers furnish clear-cut evidence as to the effect upon the size of the experimental error of changing environmental conditions. Those are the 64 steers from the Mandan grazing trials in October-November, 1922, and 72 steers from the Mandan grazing trials in October-November, 1923. Both lots were shipped from Mandan to West Fargo, unloaded, driven about 4 miles to the college pens, and weighed directly, having been subject to the excitement and shrinkage of shipment and having been without feed and water for more than 24 hours when the first day's weights were taken. The 64 steers in 1922 were weighed for 6 days, and the experimental error for the whole 6 days (315 degrees of freedom) was 17.7 pounds, as given in Table 5. However, if the first day's weights are discarded, the experimental error for the last 5 days (252 degrees of freedom) is only 12.6 pounds. Likewise, with the 72 head weighed for 8 days in 1923 the experimental error, which is 16.1 pounds when all 8 days are included, falls to 12 pounds when only the last 6 days are included. These facts show clearly that



the shrinkage in shipment did not affect all the steers equally and that a comparison of the weights of different animals, or different groups of animals directly off the cars would have been less accurate than a comparison of later weights.

The six steers in the North Dakota 1921 grazing sweet-clover trials furnish further evidence that animals whose weights are to be compared should be given the same treatment and the same ration for several days before the weights are taken. The four Herefords in this trial were rather thin and had been kept on a light ration for several days prior to May 11, when they were turned out on sweet-clover pasture. At the last moment it was decided that the sweet-clover pasture was so rank that two more steers would be required to keep it grazed down properly. The two Shorthorn steers selected for this use were in fair flesh and had been on a fairly good grain ration until the very day the grazing was started. Consequently, these two lost very much weight just at first, while the four Herefords lost little and began to gain very soon. One of the Shorthorn steers lost heavily at first, but adjusted himself to the new diet in two or three days; the other one lost very heavily at first and did not really begin to gain until about three weeks had elapsed. A measure of the difference in the reaction of the Hereford and the Shorthorn steers to the new ration (sweet-clover pasture) is afforded by the experimental error for the first six days, May 12-18. The error is 12.7 pounds when only the Herefords are included, but is 20.6 pounds when the Shorthorns with their more difficult adjustment to the new ration are included.

The West Virginia steers in the fall of 1924 were driven a distance of 6 miles after the first day's weighing, and the unequal effects of this upon different steers is clearly apparent in the variations in weight and in the three large experimental errors for these steers (13.7, 14.5, and 16.9 pounds). Probably other variations in treatment not recorded in the notes of the experiments will explain a portion of the experimental error in other cases where it is large.

It seems clear from these data that any change in environmental conditions, even though it might seem to apply to all animals equally, is apt to affect some much more than others, and consequently is to be avoided as far as possible in experimental work. It is highly desirable for this as well as other reasons that cattle should be accustomed to their lots, their water, and to the approximate ration upon which they will be fed for at least two or three days before the first weights are taken. It may sound all right for an investigator to excuse a disturbing circumstance in the treatment of his cattle by saying that all animals were subject to the same treatment. But the real question is, Did they all react to it in the same way and equally? The evidence seems clearly to indicate that they usually do not.

Weights of cattle on pasture are not necessarily inaccurate, as witness the low errors of the Mandan steers in May, 1923, and the West Virginia cattle in the fall of 1923, but there is probably more opportunity for disturbing some of the cattle more than others when they must be rounded up from a pasture than when they are merely turned out of a feed lot into the scale pen. Also it is probable that cattle on pasture do not so nearly take all their fill of feed and

water at the same hour of the day as do cattle full fed in dry lots. This may conceivably contribute a little to the inaccuracy of pasture weights for purposes of comparison.

#### PRACTICAL APPLICATION OF THESE RESULTS TO FEEDING TRIALS

The practical application of the findings of the usual feeding trial is based upon the assumption that the observed differences in gains are caused by the observed differences in feed consumption. That this assumption may not be absolutely true has long been realized. One popular way of avoiding the possibility of error arising from this assumption is to repeat the trial two or more times. Another device not yet very extensively employed is the use of duplicate lots in the same experiment. Other aids are the use of longer feeding periods, larger numbers of animals in the lots, three-day average weights instead of single weights for calculation of gains, refinements in methods of collecting and analyzing waste and refused feeds, etc.

The effect of these different refinements in experimental technic may be better understood if the sources of possible error in the fundamental assumption (that the observed differences in gains are caused by the observed differences in feed consumption) are understood clearly and are measured, as far as possible. Laying aside all questions as to the accuracy of the records of the composition and quantity of the feed consumed and wasted, there is left the question of the accuracy of the observed gains in live weight. Even if the accuracy of the observed gains is granted, there remains the very great question whether the observed differences in gain were caused by observed differences in the ration or were only coincidental with those differences.

This study bears only on the accuracy of the observed weights and of the gains calculated from them. It tells nothing about whether the two groups of steers being compared were in actual fact as evenly matched in their ability to utilize feed as the investigator assumed they were when he finished balancing his lots at the beginning of the experiment. The results of this study showed that the standard deviation of a single weight from its true value was usually from 6 to 12 pounds, being less with younger smaller cattle under absolutely uniform conditions and more with larger older cattle and where there was some disturbance in weather or other environmental condition. Stated in terms of "probable error," this means that in giving a single weight of a single steer under a given set of environmental conditions it should be understood that the actual weight is followed by a probable error of about 4 to 8 pounds.

How large, then, is the probable error of an observed gain in the weight of a steer? If his initial weight and his final weight were each single-day weights, the probable error of the gain would be the square root of the sum of the squares of the two probable errors, or approximately 6 to 11 pounds. If initial and final weights were each the average of three-day weights, these figures would be divided by the square root of 3 and would be approximately 3.3 to 6.5 pounds. Both these calculations are based upon the assumption that environmental conditions would be the same on all weighing days. Since that can not be known in advance and environmental differences can be only partially controlled, it is usually not the absolute gain in

weight of one lot that is studied but the difference between the gains made by the experimental lot and the gains made by the control lot. This removes the necessity for the assumption that environmental conditions are absolutely the same on all weighing days, but it introduces the complication that one is now interested in the probable error of a difference between two gains rather than in the probable error of one gain. Since the errors in weighing are uncorrelated with each other, the probable error of the difference between two gains is equal to the square root of the sum of the squares of the probable errors of the two gains. Thus for two steers each weighed three days at the beginning and three days at the end of the experiment, the probable error of the difference in gain due solely to inaccuracies of weights would be from 4.7 to 9.2 pounds. If there were more than one steer in each lot, as there almost always is, the error due to inaccuracy of weights would be reduced in proportion to the square root of the number of steers in a lot. Thus in the ordinary comparison of the average total gains made by two lots each containing 10 steers, the probable error due solely to inaccuracy in weights would be from 1.5 to 2.9 pounds. Even if there were 25 steers in each lot, these figures would only be reduced to 1 and 1.8 pounds.

With errors of this magnitude in the accuracy of the weights, it should be obvious that differences of less than 5 pounds in the average total gain of two lots of 10 steers are without significance, as they can easily be the result of the intrinsic inaccuracy of cattle weights. It should also be obvious that no accuracy is gained by carrying average weight or average total gain figures into decimal places in publications. Certainly bulletins giving the results of feeding trials would be easier to read if these meaningless decimals were left out of the tables. This is an improvement which could be made in the bulletins of a great many stations. For example, in Texas Bulletin No. 309 (5, p. 13), the total gain of lot 3 is given as 386.5 pounds and the total gain of lot 4 is given as 289.1 pounds. Now, the results of the present study (in which the experimental error was found to be 11.5 pounds in the initial weights and 15.2 pounds in the final weights of these particular steers) make it obvious that, so far as the accuracy of the weights are concerned and assuming identical environmental conditions at the initial and the final weighings, the real gain of lot 3 was  $386.5 \pm 2.2$  pounds and the real gain of lot 4 was  $289.1 \pm 2.5$  pounds. With probable errors as large as these, no accuracy would have been sacrificed had the gains been given as 386 and 289 pounds, and very little accuracy would have been sacrificed had the gains for lot 3 been given as "between 380 and 390 pounds" and those for lot 4 as "a little less than 290 pounds." The latter form of statement would have been accurate enough for the popular edition of the bulletin and would certainly have been more easily read and understood by feeders than the table as it was actually presented. The former statement (386 and 289 pounds) would be preferable for circulation among other animal husbandry workers, as it complies with Kelley's rule (6) for determining how many figures are significant enough for publication.

Another example of a bulletin containing tables from which meaningless decimals might have been dropped is Texas Bulletin

No. 363 (4) which reports the results of the Big Spring tests. In Table 9, for instance, the average total gains for the three lots (basis of feed-lot weights) are given as 378.86 pounds, 310.75 pounds, and 373.55 pounds. Now, the errors actually found in the accuracy of single weights in this test were 5.8 pounds for the initial weights and 8.6 pounds for the final weights, and there were 15 steers in each lot. This means that the average gains, so far as the accuracy of the weights is concerned, really were:

Lot 1,  $378.86 \pm 1.04$  pounds.

Lot 2,  $310.75 \pm 1.04$  pounds.

Lot 3,  $373.55 \pm 1.04$  pounds.

No accuracy whatever would have been sacrificed by rounding off the second decimal place and practically none by rounding off the first decimal place, although the first decimal place is significant according to a strict interpretation of Kelley's rule. The insignificant decimal places were dropped in the summary in Tables 13 and 14, and the bulletin is thereby made simpler and its findings easier to grasp, without at the same time sacrificing accuracy. These examples (which might be multiplied manifold from the publications of many of our experiment stations) and the findings of this study seem to warrant the statement that in reports of feeding trials with cattle the author who publishes average weights or average total gains carried out into decimal places does not thereby add accuracy to his findings.

In the two examples given above the actual probable errors found were used to calculate the probable error of the accuracy of the total gain. Other investigators can likewise figure the actual error in the weights with which they are working, or they can use the rough rule developed from the findings of this study, namely, that the probable error of the accuracy of a total gain will rarely, if ever, be as low as 1 pound or as high as 3 pounds with lots of steers containing the usual numbers (10 to 15) of animals. The probable error of the accuracy of the average total gain in the usual lot will ordinarily be from 1.5 to 2.5 pounds.

It will be noticed that the writers have constantly referred to the errors discussed in this study as errors in the accuracy of the weight. This is quite different from the probable error of an average weight or an average gain as calculated by the ordinary biometrical formula. The latter is an error of random sampling which assumes that the observed weights are accurate, and is intended to measure the errors which arise from the fact that the steers in any one lot do not all gain the same amount, and therefore that one lot may by "chance" contain a larger proportion of high-gaining steers than another lot does. The application of this ordinary formula to the usual feeding trial is not legitimate, however, because it is based upon the assumption that the steers have been divided into lots at random. Its application is justified only when the man who divides the steers into lots at the beginning of the experiment does so purely at random or when he can see no difference in any of the steers—i. e., when all the steers are alike in gaining ability, so far as the man who divides them can estimate. In actual practice neither of these conditions is fulfilled. The steers are divided so that in the opinion of the man who does the dividing all the lots are equally balanced. A good

steer in lot 1 is balanced by a good one in lot 2, and an inferior one in lot 1 requires that an equally inferior one be selected for lot 2, etc. Such practice in balancing the lots would eliminate all the error of random sampling if the man who balanced the lots had perfect judgment, and if he balanced them with attention only to the gaining ability of the steers, and if he had plenty of steers from which to choose. Not one of these conditions is usually fulfilled completely. The net result is that the actual error of random sampling is less than that given by the ordinary biometrical formula, but is not entirely eliminated. Just how to measure the effect of balancing on reducing the size of the real error of sampling has not, so far as the writers are aware, been worked out. The fact that balancing reduces the error of random sampling was not appreciated when the probable errors in Texas Bulletin 309 (5, p. 15) were calculated, nor does it seem to have been appreciated when the extensive study in Illinois Bulletin 165 (9) was published. It has, however, often been discussed informally in the last few years by various men interested in applying statistical methods to problems of animal production.

At all events, the error by which to judge the significance of a difference in gains will be less than the square root of the sum of the squares of the error given by the ordinary formula for the error of random sampling and the square of the error of accuracy, but it can not be less than the error of the accuracy of the weights, which is the subject of this study. This study, therefore, establishes the lower limits below which the probable error of a difference in gain can not fall.

Under uniform environmental conditions the error of the accuracy of an average weight varies inversely with the square root of the number of weights on which the average weight is based. Thus if it is impractical to weigh for 3 days, the same accuracy can be secured by using three times as many cattle and weighing only 1 day. Thus the accuracy of the average weight of 30 steers for 1 day should be equal to that of the average weight of 10 steers for 3 days. However, on the one hand, the 30 steers weighed 1 day would have a considerably smaller error of sampling. On the other hand, the 10 steers weighed for 3 days would permit an estimation of the effect of environmental condition and therefore a more accurate estimation of the actual weight of gain of the steers in pounds. This advantage of the 3-day weight is not of importance in comparisons between lots fed at the same time and weighed on the same days, but it would be an advantage in attempts to use the absolute gain in calculating the energy content of the feed or for comparison with gains on similar rations in other years or at other places. Thus a 1-day weight of 30 steers and a 3-day weight of 10 steers are not exactly identical in value for experimental purposes. Each has its advantages, although the probable error of their accuracy for purposes of comparison with similar lots in the same experiment would be identical. If one has a given number of steers and wishes to know what accuracy he will gain by weighing for 3 days instead of 1 day, the findings of this study confirm those published in the preliminary report—that he will reduce the error of the accuracy of his weights for purposes of comparison with other lots in the same experiment to only 58 per cent ( $\sqrt{\frac{1}{3}}$ ) as much as it would have been with a 1-day weight, and,

in addition, he will have a still better estimate of the accuracy of the absolute weights and gains for purposes of comparison with weights and gains made in other years or at other places.

### CONCLUSIONS

The experimental error (standard deviation) in the accuracy of single weights of cattle may be expected ordinarily under uniform conditions to be between 6 and 12 pounds.

The experimental error is somewhat smaller with younger cattle, and is distinctly smaller under unchanging environmental conditions than where some sudden change is made in some important condition between two of the weighings.

The size of the experimental error is probably not directly related to the gross weight of the cattle, except in so far as both are partially determined by age. The size of the experimental error may perhaps be rather closely related to the fat-free weight of the animal or to the size and capacity of the animal's digestive tract.

Breed seems to have little or no effect upon the size of the experimental error.

Nervousness and excitability of the cattle seem to have little or no effect upon the size of the experimental error when all the steers in a lot are excitable or when all are gentle. Including excitable and gentle animals in the same lot does increase the experimental error.

Weighing cattle to the nearest 5 pounds sacrifices a considerable amount of information. Weighing to the nearest 2 pounds does not lead to any serious loss of information, except with really small cattle. Weighing to the nearest pound adds some accuracy when working with calves and small yearlings. Weighing to units smaller than 1 pound does not seem to possess any advantage which would make the practice worth adopting.

The "probable error" of the accuracy of a single weight under uniform conditions is approximately equal to two-thirds of what has been called in this paper the "experimental error"—i. e., the probable error of a single weight is usually 4 to 8 pounds. The probable error of the accuracy of the average weight of a group may be obtained by dividing the above figures by the square root of the number of steers in the group. Thus, in the ordinary lot of 10 steers weighed for three successive days, the probable error of the accuracy of the average weight would usually be 4 to 8 pounds divided by the square root of 30 (the number of weights), which would be about 1 to 2 pounds. The probable error of the accuracy of a difference between the average weights of two such lots would be about 40 per cent larger, or, roughly, 1 to 3 pounds. With errors of such size no accuracy is gained by carrying into decimal places average weights for the ordinary lot of experimental cattle. It is just as accurate to carry the average weight only to the nearest whole pound, and such a practice would certainly add to the ease with which the published results might be read.

The "probable errors" and "experimental errors" studied in this paper refer only to the accuracy of the weight of the cattle composing those lots, and do not have any bearing on the probable error of the sampling which took place when the animals were selected for those lots. The latter is the probable error given by the ordinary

biometrical formula. Unfortunately, the ordinary formula is not usually applicable to feeding experiments because sampling is not random. The true figure by which to judge the statistical significance of a difference in gains can not be less than the error of the accuracy of the weight (which is the subject of this paper) nor more than the square root of the sum of the squares of the error of accuracy and the error of sampling. The absence of a satisfactory method for estimating the error of sampling which is not random does not at present permit more than a rough estimate of the real error by which to judge the statistical significance of the results of feeding trials. The upper and lower limits between which such an error lies can, however, be rather accurately estimated.

The relative error of a 1-day weight and a 3-day weight is as the square root of one and the square root of one-third when the comparison is being made between the weight changes made by the experimental lot and the weight changes made by the control lot. However, if the experimenter wishes to study absolute changes in weight (as where there is no control lot) or to compare gains made at different times or in different places, the 3-day weight has the additional advantage of permitting the estimation of the day-to-day fluctuation in weight, and hence a more accurate estimation of the absolute weights and gains. For purposes of comparison with the control lot, weighing for 2 additional days eliminates about 42 per cent of the error contained in a 1-day weight. If it is inconvenient or impossible to weigh more than 1 day, 3-day accuracy for comparison with the control lot may be attained by using three times as many animals in each lot. This procedure has the statistical advantage of giving a much smaller error of sampling, but economic reasons usually preclude its use.

#### LITERATURE CITED

- (1) ARMSBY, H. P.  
1898. THE MAINTENANCE RATION OF CATTLE. Penn. Agr. Expt. Sta. Bul. 42, 188 p., illus.
- (2) BLACK, W. H., JONES, J. M., and KEATING, F. E.  
1928. SORGO SILAGE, SORGO FODDER, AND COTTONSEED HULLS AS ROUGHAGES IN RATIONS FOR FATTENING CALVES IN THE SOUTHWEST. U. S. Dept. Agr. Tech. Bul. 43, 24 p., illus.
- (3) FISHER, R. A.  
1925. STATISTICAL METHODS FOR RESEARCH WORKERS. 239 p., illus. Edinburgh and London, Oliver and Boyd.
- (4) JONES, J. M., BLACK, W. H., and KEATING, F. E.  
1927. SORGO SILAGE, SORGO FODDER AND COTTONSEED HULLS AS ROUGHAGES IN RATIONS FOR FATTENING CALVES. Tex. Agr. Expt. Sta. Bul. 363, 36 p., illus.
- (5) ——— LUSH, J. L., and JONES, J. H.  
1923. I. FATTENING STEERS ON COTTONSEED MEAL AND HULLS WITH AND WITHOUT CORN. II. THE INFLUENCE OF AGE ON FATTENING STEERS. Tex. Agr. Expt. Sta. Bul. 309, 31 p., illus.
- (6) KELLEY, T. L.  
1924. HOW MANY FIGURES ARE SIGNIFICANT? Science (n. s.) 60: 524.
- (7) LUSH, J. L., and BLACK, W. H.  
1927. HOW MUCH ACCURACY IS GAINED BY WEIGHING CATTLE THREE DAYS INSTEAD OF ONE AT THE BEGINNING AND END OF FEEDING EXPERIMENTS. Amer. Soc. Anim. Prod. Proc. 1925/26: 206-210.
- (8) ——— and HALE, F.  
1927. RICE BRAN AS A FEED FOR DAIRY COWS. Tex. Agr. Expt. Sta. Bul. 352, 22 p., illus.

- 
- (9) MITCHELL, H. H., and GRINDLEY, H. S.  
1913. THE ELEMENT OF UNCERTAINTY IN THE INTERPRETATION OF FEED-  
ING EXPERIMENTS. Ill. Agr. Expt. Sta. Bul. 165, p. 463-579,  
illus.
- (10) SHEETS, E. W., and TUCKWILLER, R. H.  
1926. EFFECT OF WINTER RATIONS ON GAINS OF CALVES MARKETED AS  
3-YEAR-OLD STEERS. U. S. Dept. Agr. Bul. 1431, 11 p., illus.
- (11) SHEPPERD, J. H.  
1927. SWEET CLOVER EXPERIMENTS IN PASTURING. N. Dak. Agr. Expt.  
Sta. Bul. 211, 56 p., illus.
- (12) WILSON, C. V., SHEETS, E. W., and TUCKWILLER, R. H.  
1928. EFFECT OF WINTER RATIONS ON GAINS OF CALVES MARKETED AS  
3-YEAR-OLD STEERS. W. Va. Agr. Expt. Sta. Bul. 218. (In  
press.)





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## INHERITANCE OF EARLINESS AND OTHER AGRONOMIC CHARACTERS IN RICE<sup>1</sup>

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### INTRODUCTION

Rice growing in California on a commercial scale began in 1912. The leading commercial variety grown during the first five years was Wataribune, a late-maturing, short-grain rice. This variety later was replaced by early and midseason short-grain varieties. Some of these varieties were introductions from Japan, while others were pure-line selections distributed from the Biggs Rice Field Station.

In California, early and midseason rices, especially the early-maturing varieties, do not yield as well, as a rule, as the late-maturing varieties. However, the danger of loss of crop due to wet weather in the fall largely is eliminated by the use of early-maturing rices. For this reason there is a constant demand for early-maturing rice varieties that will yield and mill well. Many early varieties are available, but those which have been tested in California do not yield as well, except on rich soil, as do the midseason and late-maturing varieties.

There have not been reported, so far as can be found, any studies in the United States on inheritance in rice, though the crop has been grown in this country since 1694.

The work reported in this paper began in 1922. A cross was made by the writer in that year, using an early-maturing variety, Niro Vialone, as the female parent, and a midseason variety, Caloro, as the male parent. The cross was made to determine whether by recombination of characters in succeeding generations it would be possible to secure a higher-yielding early-maturing variety than those which were available. A study of the inheritance of several contrasted characters other than earliness was made in the  $F_2$  and  $F_3$  generations.

### MATERIAL AND METHODS

The most accurate index of earliness in rice is the appearance of the upper spikelets of the panicle above the leaf sheath. This index of earliness was found to be more satisfactory than a record of the date when the plant is fully headed or the kernels are all ripe. "Fully headed," as used in this study, means that all panicles on a plant were

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exserted from the sheaths. "All ripe" means that the kernels on the lower part of the panicle were in the stiff-dough stage, the kernels of the upper spikelets were hard, and the lemmas were straw colored.

Niro Vialone, the female parent, is an early-maturing variety with many organs purplish in color. The average date of maturity for this variety during the six-year period from 1918 to 1923, inclusive, was September 14. It normally is awnless, but occasionally a purplish awn, varying in length from 1 to 2 mm., may be observed. The lemma tips, the glumes, the stigmas, the pulvinuses, the bases of the ligules, the auricles, the leaf sheaths, the leaf blades, the nodes, and the internodes are purplish in color. The upper portion of the peduncle or internode which supports the panicle often is called the "neck." In Niro Vialone the neck is sinuous instead of straight, as in most varieties.

Caloro, the male parent, is a midseason normally green variety. The average date of maturity for this variety during the six-year period from 1918 to 1923, inclusive, was October 4. Normally about one-half the spikelets on a panicle are awnless, but the remaining spikelets of Caloro have white awns which are variable in length. The plant organs which are described as colored in the Niro Vialone variety are green or uncolored in Caloro. The peduncle or neck is straight.

#### INHERITANCE OF EARLINESS

In making the cross all but about 20 spikelets were clipped from the panicle of the female parent plant. The remaining spikelets were emasculated one morning and pollinated on the following day with pollen collected at random from plants of the male parent. Two crossed seeds were obtained.

#### F<sub>1</sub> PROGENY

The two crossed seeds were sown in 1923, but unfortunately one failed to germinate. The F<sub>1</sub> plant secured was bagged during the blossoming period to avoid the possibility of cross-fertilization. In date of maturity the F<sub>1</sub> plant was intermediate, but nearer the early parent.

It may be of interest to include here data on the dates of first spikelet emergence of F<sub>1</sub> hybrids from other rice crosses that were grown at Biggs in 1925.

On May 13, 1925, F<sub>1</sub> seed from nine crosses and seed of all parent varieties were sown in paper pots, one seed in each pot. After the seedlings had emerged the bottoms of the pots were removed and the seedlings were transplanted in rod rows spaced 2 feet apart. The seedlings were spaced about 1 foot apart in the rows. The plants, therefore, had ample room for development.

Notes were taken on the date of first spikelet emergence for each individual F<sub>1</sub> and parent plant. First spikelet emergence, as used in this paper, means that two or more spikelets of the earliest panicle were showing above the leaf sheaths. In Table 1 are presented the data on the average date of first spikelet emergence for the nine crosses and the parent varieties.

TABLE 1.—Average date of first emergence of spikelets of the parent varieties and the  $F_1$  plants from nine rice crosses grown at Biggs, Calif., in 1925

Female parent			Male parent			$F_1$ progeny	
Name	Number of plants	Average date of emergence of first spikelets	Name	Number of plants	Average date of emergence of first spikelets	Number of plants	Average date of emergence of first spikelets
C. I. No. 5346.....	5	Aug. 15	Italian red.....	6	Aug. 10	7	Aug. 9
Niro Vialone.....	7	Aug. 5	Wataribune.....	5	Sept. 4	2	Aug. 29
Caloro.....	6	Aug. 28	Viola.....	7	Aug. 15	7	Aug. 27
Colusa.....	5	Aug. 13	Italian red.....	6	Aug. 10	3	Aug. 18
Do.....	5	do.	Lady Wright.....	7	Aug. 22	29	Aug. 28
Lady Wright.....	7	Aug. 22	Caloro.....	6	Aug. 28	30	Aug. 29
Caloro.....	6	Aug. 28	Lady Wright.....	7	Aug. 22	23	Aug. 28
Wataribune.....	5	Sept. 4	Red rice.....	5	Aug. 23 <sup>a</sup>	6	Oct. 16
Caloro.....	6	Aug. 28	C. I. No. 5315.....	5	Aug. 21	33	Oct. 19

<sup>a</sup> In 1926.

For the cross C. I. (Cereal Investigations) No. 5346  $\times$  Italian red, the  $F_1$  plants began to head earlier than those of either parent. For the crosses Niro Vialone  $\times$  Wataribune and Caloro  $\times$  Viola the  $F_1$  plants were nearer the late parent in this character. For the cross Lady Wright  $\times$  Caloro and its reciprocal, the  $F_1$  plants were about the same as the late parent. For the crosses Colusa  $\times$  Italian red and Colusa  $\times$  Lady Wright, the  $F_1$  plants were considerably later than the late parent. For the crosses Wataribune  $\times$  red rice and Caloro  $\times$  C. I. No. 5315, the  $F_1$  plants were 42 and 52 days later, respectively, than the late parent in first emergence of spikelets. No mature seed was produced by  $F_1$  plants of either of these two crosses. Yet, in rows adjacent to the  $F_1$  plants Wataribune matured on October 18 and red rice on October 4 (1926), Caloro on October 9, and C. I. No. 5315 on October 5.

Both red rice and C. I. No. 5315 have wider leaves, tiller more freely, and shatter much more readily than do the Caloro and Wataribune. These two first-named varieties also have a spreading habit of growth and rather open panicles, whereas Caloro and Wataribune are erect in growth and have panicles of average compactness. This unexpected behavior of the  $F_1$  plants in these two crosses suggests that the red rice and C. I. No. 5315 varieties may belong to a different species from Caloro and Wataribune, which are Japanese varieties.

The results presented here indicate that in rice  $F_1$  plants from various crosses may be earlier than the early parent, later than the late parent, or nearer the early or the late parent in time of first emergence of spikelets. The parents also may be so different that the  $F_1$  plants are sterile.

#### $F_2$ PROGENY

Hoshino (5)<sup>3</sup> found in a cross between early and late-maturing rice that the time of flowering in  $F_1$  was intermediate but nearer that of the early parent. In  $F_2$  segregation was complex, but no transgressive inheritance occurred. In  $F_3$  some families showed results very much like those from the parents. The author believed that the inheritance of earliness in this case could be explained on a three-

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 601.

factor hypothesis. Ikeno (6) reports that, in crosses of early and late rice, the  $F_1$  was intermediate and segregation in  $F_2$  was complex and apparently due to multiple factors. Hector, according to Evans (4), found that the  $F_2$  progeny from a cross between a colored autumn (early) rice and an uncolored winter (late) rice segregated into two distinct groups with respect to date of flowering. These two flowering periods were nearly the same as the flowering dates of the two parents, with an interval of about three weeks during which time no blooming occurred. The ratio of early to late plants was approximately 1:3. Bhide (2) found that lateness was dominant over earliness in two varieties with which he worked, causing ratios for the cross and its reciprocal of approximately 2.84 and 2.75 late plants to 1 early, respectively. But in other crosses the dominance of lateness over earliness was not very evident.

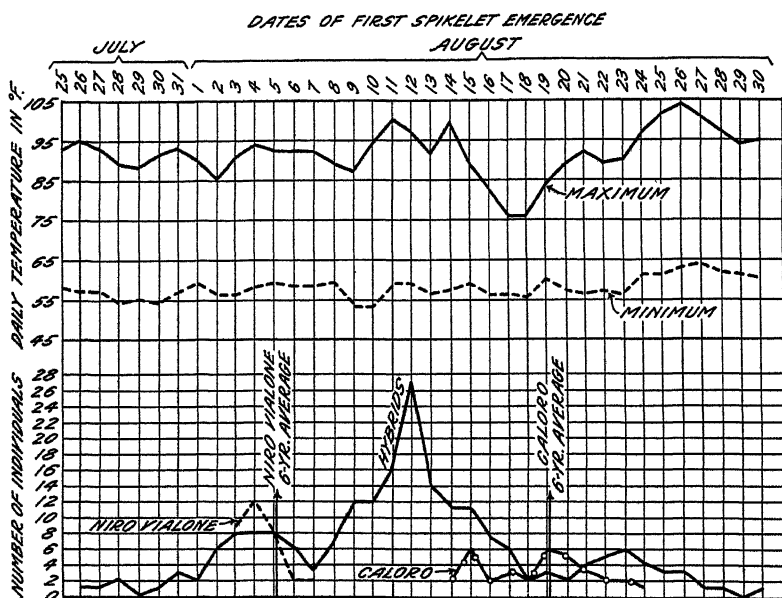


FIG. 1.—Frequency polygon showing dates of first spikelet emergence for the plants of the  $F_2$  progeny and parent varieties of the Niro Vialone  $\times$  Caloro rice cross grown at Biggs, Calif., with the daily maximum and minimum temperatures during the period

Nomura and Yamazaki (7) found that the  $F_1$  hybrids were a few days later in shooting than was the late parent, and in  $F_2$  segregation was in the ratio of about 3 late to 1 early plant. The late plants on an average were slightly later and the early plants slightly earlier than the late and early parents, respectively. The  $F_3$  progeny from the  $F_2$  early type showed the monomodal narrow variation. The  $F_2$  late type produced two groups of plants, one showing the narrow monomodal variation, while the other segregated again into about 3 late to 1 early plant. The authors reached the conclusion that, with respect to factorial composition, the rices studied were as follows:

Cross No. 1 AAbbcc  $\times$  aabbCC (early  $\times$  late), cross No. 2 aabbCC  $\times$  aaBBcc (late  $\times$  early). When all these factors are present the shooting time was found to be earlier, 91 (1921, 1922) or 100 days (1923). Each of the factors A, B, and C prolongs the shooting time to a certain extent, C being most and B least

efficacious in this respect. By the combination of either A and C or B and C the shooting time becomes longer.

On May 9, 1924, the seeds obtained from the  $F_1$  plant of the cross Niro Vialone  $\times$  Caloro was sown on river-bottom soil in tubs. The  $F_2$  plants emerged on May 18 and, when about 6 inches high, were transplanted to the nursery in rows 17 feet long and 3 feet apart. The plants were spaced about 6 inches apart in the rows. The parent material, grown for comparative purposes, likewise was sown in tubs and transplanted at the same time as the  $F_2$  plants.

The index of earliness used in this study was the time of appearance of the first spikelet above the leaf sheath. This datum can be more accurately taken than others that might be used in a study of this nature with rice. The date of spikelet emergence, as used in this paper, therefore, means the appearance of the first spikelet above the leaf sheath.

In the cross Niro Vialone  $\times$  Caloro the  $F_1$  plant was intermediate in date of spikelet emergence, though slightly nearer the early parent. The frequency distribution of the plants of the Niro Vialone and Caloro parents and of the  $F_2$  progeny is shown in Table 2, and a frequency polygon is given in Figure 1.

TABLE 2.—Frequency distribution of the parents and  $F_2$  progeny of the Niro Vialone  $\times$  Caloro rice cross by dates of first spikelet emergence of plants at Biggs, Calif., in 1924

Date of emergence of first spikelet	Parents and progeny (number)			Date of emergence of first spikelet	Parents and progeny (number)		
	Niro Vialone	Caloro	$F_2$ progeny		Niro Vialone	Caloro	$F_2$ progeny
July 26.....			1	Aug. 14.....		2	11
July 27.....			1	Aug. 15.....		6	11
July 28.....			2	Aug. 16.....		2	7
July 29.....			0	Aug. 17.....		3	6
July 30.....			1	Aug. 18.....		2	2
July 31.....			3	Aug. 19.....		6	3
Aug. 1.....			2	Aug. 20.....		5	2
Aug. 2.....			6	Aug. 21.....		3	4
Aug. 3.....	9		8	Aug. 22.....		2	5
Aug. 4.....	12		8	Aug. 23.....		2	6
Aug. 5.....	7		8	Aug. 24.....		1	4
Aug. 6.....	2		6	Aug. 25.....			3
Aug. 7.....	2		3	Aug. 26.....			3
Aug. 8.....			7	Aug. 27.....			1
Aug. 9.....			12	Aug. 28.....			1
Aug. 10.....			12	Aug. 29.....			0
Aug. 11.....			16	Aug. 30.....			1
Aug. 12.....			27				
Aug. 13.....			14	Total.....	32	34	201

Inspection of Table 2 shows that a number of  $F_2$  plants began to exert the upper spikelets earlier than the earliest of the early-parent plants, and likewise a number of  $F_2$  plants began to exert the uppermost spikelets later than the latest of the late-parent plants. This shows transgressive inheritance.

The frequency polygon shown in Figure 1 is trimodal, with a small early group, a large intermediate group, and a small midseason group of plants. The earliest  $F_2$  plant began to exert flowers eight days earlier than the earliest early-parent plant, and the latest  $F_2$  plant began to flower six days later than the latest late-parent plant. It is possible that the lowering of the maximum temperature from

August 16 to August 19, inclusive, may have caused the low frequency of flowering on these dates. Akemine (1) found that rice flowers will open at a minimum temperature of 59° F. He also found, however, that the maximum of opening is at temperatures varying from 95° to 104° F., and that the higher the natural temperature the more active is the process of blooming.

If it is assumed that the depression in flowering observed from August 16 to August 19 was due to the lowering of the maximum daily temperature at this time, which is not improbable, the  $F_2$  progeny would then be distributed into two groups, an early and a midseason one extending well beyond the midseason parent in time of spikelet exertion. By using August 7, the date by which all the early-parent plants had begun to exert spikelets, as the limit classes of the two and assigning two of the plants which began to flower on this date to the early group and one to the midseason group, there then would be 48 plants in the early group and 159 plants in

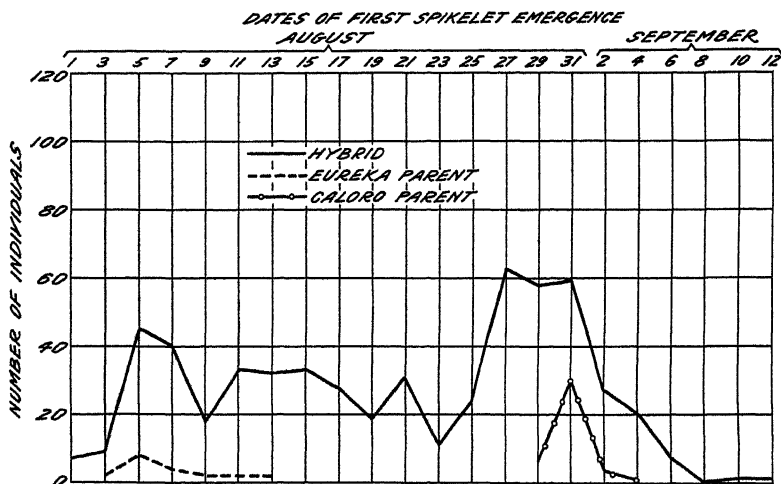


FIG. 2.—Frequency polygon showing dates of first spikelet emergence for the plants of the  $F_2$  progeny and parent varieties of the Eureka x Caloro rice cross grown at Biggs, Calif., in 1925.

the midseason group. This is approximately 3 midseason plants to 1 early, which suggests a single-factor difference for earliness. With this grouping the deviation from the 3:1 ratio is  $3.75 \pm 4.20$ , and is not significant.

Again, if the  $F_2$  population is divided into early and midseason groups by using the modal frequency class (August 12) as the dividing line and assigning one-half of the plants in this modal class to each group, there are 109 plants in the early group and 98 plants in the late one. This indicates a 1:2:1 ratio with most of the  $F_2$  plants intermediate in blooming time between those of the parents.

By using August 7 and August 18 as group limits, and distributing individuals flowering on August 7 as indicated in the second preceding paragraph, which gives 48 early, 126 intermediate, and 33 late plants, there are 23.2 per cent of the plants in the early group, 60.9 per cent in the intermediate group, and 15.9 per cent in the late group. These percentages indicate that, with respect to earliness, the  $F_2$  progeny at least suggests a 1:2:1 ratio of early, inter-

mediate, and midseason groups, respectively. These ratios, however, are only suggestive, for the transgressive inheritance in this case indicates that more than a single-factor difference is involved in the production of earliness.

The date of first spikelet emergence also was studied in  $F_2$  plants from the cross Eureka  $\times$  Caloro, grown in 1925. Eureka is an early-maturing variety. The  $F_1$  in this cross was intermediate, but nearer the late parent. In  $F_2$ , transgressive segregation for date of first spikelet emergence occurred, as is shown in the frequency distribution presented in Table 3 and the frequency polygon in Figure 2. There were 184  $F_2$  plants that began to produce spikelets as early as or earlier than the latest early-parent plant; 243  $F_2$  plants that began spikelet emergence between the dates of spikelet emergence on the latest early-parent plant and on the earliest late-parent plant; and 138  $F_2$  plants that were as late as or later than the range of the late-parent plants. Thus there was a heaping up of plants in the intermediate position, which was also observed to be the case in the  $F_2$  population from the cross Niro Vialone  $\times$  Caloro. In both of these crosses there were many more midseason and late than early plants in the  $F_2$  segregations.

TABLE 3.—Frequency distribution of the parents and  $F_2$  progeny of the Eureka  $\times$  Caloro rice cross by dates of first spikelet emergence in plants at Biggs, Calif., in 1925

Date of emergence of first spikelet	Parents and progeny (number)			Date of emergence of first spikelet	Parents and progeny (number)		
	Eureka	Caloro	$F_2$ progeny		Eureka	Caloro	$F_2$ progeny
July 31.....			1	Aug. 23.....			5
Aug. 1.....			6	Aug. 24.....			1
Aug. 2.....			3	Aug. 25.....			23
Aug. 3.....	2		6	Aug. 26.....			23
Aug. 4.....	4		29	Aug. 27.....			40
Aug. 5.....	4		16	Aug. 28.....			35
Aug. 6.....	4		28	Aug. 29.....		7	23
Aug. 7.....	3		28	Aug. 30.....		24	35
Aug. 8.....	1		11	Aug. 31.....		6	24
Aug. 9.....	2		4	Sept. 1.....		2	18
Aug. 10.....			14	Sept. 2.....		1	9
Aug. 11.....	2		19	Sept. 3.....			12
Aug. 12.....			14	Sept. 4.....		1	5
Aug. 13.....	2		12	Sept. 5.....			1
Aug. 14.....			20	Sept. 6.....			6
Aug. 15.....			10	Sept. 7.....			0
Aug. 16.....			23	Sept. 8.....			0
Aug. 17.....			9	Sept. 9.....			1
Aug. 18.....			18	Sept. 10.....			0
Aug. 19.....			8	Sept. 11.....			1
Aug. 20.....			11				
Aug. 21.....			17	Total.....	20	41	565
Aug. 22.....			14				
			6				

#### $F_2$ PROGENY

In 1925, 47  $F_3$  families were grown from the cross Niro Vialone  $\times$  Caloro, including early, midseason, and late progenies. These consisted of 18 families with purple leaves, 14 with green leaves, 9 with purple-striped leaves, and 6 with green leaves and red lemma tips. Each family was grown in four row rows spaced 2 feet apart, and the plants were spaced about 6 inches apart in the rows. Notes were taken on the date of first and last spikelet emergence within each family. In nine families the date of first spikelet emergence

was taken for each plant, as well as the number of culms per plant, height of plant, length of panicles, and yield per plant. These 47 families also were used in a study of the inheritance of color and other contrasting characters.

TABLE 4.—Frequency distribution of the parents and  $F_3$  progeny of the Niro Vialone  $\times$  Caloro rice cross by dates of first spikelet emergence in plants at Biggs, Calif., in 1925

Date of emergence of first spikelet	Parents and progeny (number)											Total progeny
	Niro Vialone	Caloro	221A22	A41	A59	A85	A14	A60	A50	A170	A12	
July 30.			2	1	2							5
July 31.			2	1	1	1						5
Aug. 1.			8	1	1	4	1					15
Aug. 2.			28	8	4	5	2	1				48
Aug. 3.			21	4	6	6	6		2			45
Aug. 4.			9	2	2	6	5	1	1	3		29
Aug. 5.			14	6	5	10	9	4	1	1		50
Aug. 6.	4		24	4	6	5	18	2	3	3		65
Aug. 7.	4		4	5	2	3	12	4	5	2		37
Aug. 8.	3			10	3	10	5	2	2	4		41
Aug. 9.	2		5	7	2	13	5	2	2	2		38
Aug. 10.	4		1	7	5	9	6	2	4			34
Aug. 11.			4	4	3	2	1	3		2		19
Aug. 12.	2		2	6	1	7	9			2		27
Aug. 13.			4	5	7	5	6	2	1	4		34
Aug. 14.			2	1		8	4	3	1	5		24
Aug. 15.	4				9	1	5	3		4		22
Aug. 16.	2				1	2	3			9		17
Aug. 17.	1		1	2	5	1	5	1		5		20
Aug. 18.				1	3	3	2			2		11
Aug. 19.				2	3	5	3		1	5		19
Aug. 20.					2	2	4			1		9
Aug. 21.					7	2	3	1		3		16
Aug. 22.				1	2		4			1		9
Aug. 23.				1		5		2		1		9
Aug. 24.					5	4	3		1	6		20
Aug. 25.				1	1	4		3		4		13
Aug. 26.				2	1	4	3		2	8		23
Aug. 27.				14	5	7		3	6	12	1	48
Aug. 28.				11	4	4	1	9	11	5	4	49
Aug. 29.		7		3	4	1		5	11	4	5	33
Aug. 30.		24		3	10			3	10	6	8	40
Aug. 31.		6		1	7			2	5	12	16	43
Sept. 1.		2			1			3	2	7	14	27
Sept. 2.		1			3			1		4	8	16
Sept. 3.					1			1		2	20	24
Sept. 4.		1									2	2
Sept. 5.								2			7	9
Sept. 6.									3		1	4
Sept. 7.									5		1	6
Sept. 8.									2		3	5
Sept. 9.									2		1	3
Sept. 10.									3			3
Sept. 11.									5		1	6
Sept. 12.									3		1	4
Sept. 13.									1		1	2
Sept. 14.									1		2	3
Sept. 15.											1	1
Sept. 16.									1		1	2
Sept. 17.											1	1
Total	26	41	136	114	132	135	124	69	97	129	99	1,035

The frequency distribution for date of first spikelet emergence of the nine  $F_3$  families and the parents is shown in Table 4 and the frequency polygons in Figure 3. It is shown in Table 4 that plants in eight of the nine  $F_3$  families showed emergence of spikelets earlier than did the earliest of the early parent plants, and that in three families (including the ninth) there were plants later than the latest late-parent plant. There were plants in both families A50 and A60



that began spikelet emergence before any of the early parent plants, and also some that were later in spikelet emergence than the latest of the late-parent plants.

In all nine families there was transgressive inheritance with respect to date of first spikelet emergence. Family A22 bred almost true for earliness; that is, all plants in this family began to produce spikelets as early as did those of the early parent, and some plants were earlier. Family A12 was late in maturing, and some few plants were later than the late parent. Families A85, A14, and A59, if the plants were separated into two groups, using August 17, 18, and 23, respectively, as division dates, would show ratios of approximately 3 early to 1 late plant. In the three families there are 287 early to 104 late plants, or a deviation from a 3 : 1 ratio of 6.25 plants with a probable error of  $\pm 5.78$ . In families A60, and A170, if the plants are grouped as early and late, using August 19 and 22 as the respective division dates, there are in each case approximately 9 late to 7 early plants. In family A41, when August 15 is used as a dividing date, there are 72

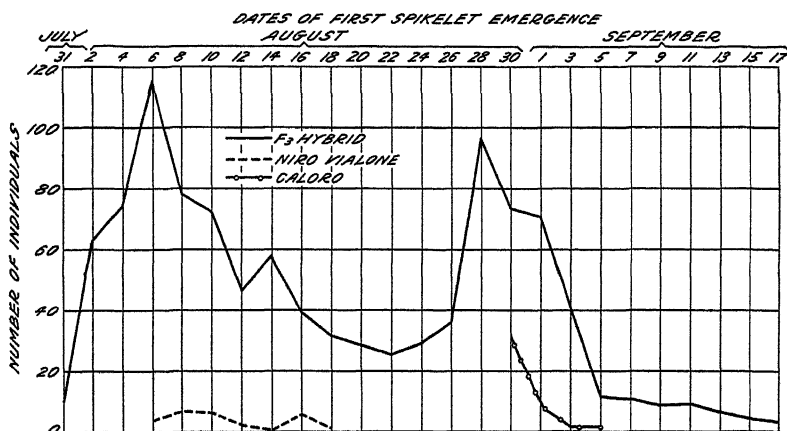


FIG. 3.—Frequency polygon showing dates of first spikelet emergence for the plants of the parent varieties and of nine  $F_3$  families of the Niro Vialone  $\times$  Caloro rice cross grown at Biggs, Calif., in 1925

early to 42 late plants, a not very close approximation of 9 early plants to 7 late.

The segregation of family A50 is distinctly into three groups in a ratio of 1 early to 2 midseason to 1 late plant. The deviation from the calculated numbers in a 1 : 2 : 1 ratio is very small for each group. This is the most distinct segregation obtained in the  $F_3$  families.

#### F<sub>4</sub> PROGENY

Selections of mostly early, but a few midseason, plants were made from 47  $F_3$  families in the fall of 1925, and 355 of these selections from 44  $F_3$  families were grown in 1926. In Table 5 are given the date of first spikelet emergence for the  $F_2$  plants grown in 1924 and the approximate date of first spikelet emergence for the  $F_3$  and  $F_4$  families grown in 1925 and 1926, respectively. In 1924 the notes are from single  $F_2$  plants; in 1925 from a family consisting of four rod rows with plants spaced approximately 6 inches apart; and in 1926 from 1 to 10 families grown in rod rows and derived from single plants selected in 1925.

TABLE 5.—Date of first spikelet emergence of  $F_2$  plants in 1924 and of first and last spikelet emergence of  $F_3$  and  $F_4$  progeny in 1925 and 1926, respectively, from the rice cross *Nuro Vialone* × *Caloro*, at Biggs, Calif.

[T=Breeding true or almost true, S=segregating for dates of maturity; T-S=both true and segregating]

Color group and No. of family	Date of first spikelet emergence of F <sub>2</sub> plants in 1924	Date of first and last spikelet emergence				Notes
		F <sub>2</sub> families in 1925		F <sub>4</sub> families in 1926		
		First	Last	First	Last	
Purple leaves:						
22A4	Aug. 2	Aug. 1	Aug. 28	July 21	July 27	T.
22A22	July 31	do.	Aug. 17	do.	do.	T.
22A43	Aug. 2	Aug. 2	Aug. 27	July 24	July 31	T.
22A61	July 28	July 31	Aug. 10	July 21	July 24	T.
22A26	Aug. 2	Aug. 2	Aug. 18	July 24	July 31	T.
22A69	Aug. 3	do.	do.	July 27	do.	T.
22A110	July 31	July 31	Aug. 19	do.	Aug. 2	T.
22A114	July 28	July 29	do.	July 21	July 24	T.
22A63	Aug. 9	July 31	Aug. 29	do.	Aug. 18	T-S.
22A80	Aug. 7	do.	Sept. 1	do.	Aug. 21	T-S.
22A83	Aug. 10	Aug. 2	do.	July 24	Aug. 18	T-S.
22A85	Aug. 5	July 31	Aug. 29	July 21	do.	S.
22A28	Aug. 8	do.	Sept. 1	do.	July 27	T-S.
22A35	do.	Aug. 2	do.	July 24	Aug. 23	T-S.
22A29	Aug. 12	Aug. 5	do.	July 28	July 31	T-S.
22A23	Aug. 13	Aug. 3	Sept. 10	do.	do.	
22A60	Aug. 11	Aug. 2	Sept. 5	do.	do.	
22A154	Aug. 16	Aug. 15	Sept. 19	Aug. 4	Aug. 25	T-S.
Green leaves:						
22A14	Aug. 3	Aug. 1	Aug. 28	July 27	do.	T
22A165	Aug. 4	Aug. 2	Aug. 18	July 24	July 28	T.
22A15	Aug. 9	Aug. 14	Aug. 30	Aug. 8	Aug. 16	T.
22A51	do.	Aug. 2	Aug. 20	July 24	Aug. 7	T
22A190	Aug. 4	Aug. 1	Aug. 17	do.	July 31	T.
22A54	Aug. 12	Aug. 7	Aug. 29	July 31	Aug. 8	T-S.
22A170	do.	Aug. 4	Sept. 3	July 28	do.	T.
22A173	Aug. 11	Aug. 6	Sept. 5	July 29	Aug. 12	T-S.
22A1	Aug. 9	Aug. 1	Sept. 14	July 27	July 31	S.
22A13	Aug. 10	Aug. 3	Sept. 1	July 28	Aug. 25	T-S.
22A37	Aug. 12	Aug. 6	Sept. 19	July 27	Aug. 5	T-S.
22A12	Aug. 15	Aug. 27	Sept. 17	do.	July 31	T.
22A42	Aug. 14	Aug. 3	Sept. 1	do.	do.	
22A140	Aug. 19	do.	Sept. 20	Aug. 25	Aug. 27	T
Purple-striped leaves:						
22A88	July 26	July 28	Aug. 17	July 21	July 27	T.
22A38	Aug. 5	Aug. 2	Sept. 1	do.	do.	T-S.
22A163	Aug. 4	Aug. 4	Aug. 17	July 24	July 31	T.
22A174	do.	Aug. 3	Aug. 19	do.	do.	T.
22A81	Aug. 6	Aug. 1	Sept. 1	July 17	Aug. 17	T-S.
22A30	Aug. 4	Aug. 4	Aug. 19	July 24	July 27	T.
22A59	Aug. 6	July 30	Sept. 3	July 21	do.	T.
22A167	Aug. 10	Aug. 5	Sept. 13	July 24	Aug. 16	T-S.
22A179	Aug. 12	Aug. 2	Sept. 1	July 31	Aug. 25	T-S.
Green leaves, red lemma tips:						
22A41	Aug. 6	July 30	Aug. 31	July 21	do.	T.
22A168	Aug. 2	Aug. 2	Aug. 13	July 27	July 31	T.
22A45	Aug. 9	do.	Sept. 9	do.	Aug. 12	T-S.
22A133	Aug. 11	do.	Sept. 14	do.	July 31	T-S.
22A50	Aug. 12	Aug. 3	Sept. 15	do.	do.	T.
22A172	Aug. 15	Aug. 28	Sept. 18	Aug. 16	Aug. 21	T-S.

The  $F_2$  plants grown in 1924 were first irrigated on May 9 and the  $F_3$  and  $F_4$  families grown in 1925 and 1926 were first irrigated on May 16 and May 7, respectively. In Table 5 it is observed that nearly all  $F_2$  plants have given rise to earlier forms in  $F_3$  and  $F_4$ , which indicates that all  $F_2$  plants in this case were heterozygous with respect to earliness.

In 1926 there was a total of 203  $F_4$  selections derived from single  $F_3$  plants that bred true or almost true for early maturity, 41 that bred true or almost true for midseason maturity, and 111 that segregated for early and midseason plants.

A number of the early-maturing plant selections made in  $F_3$  and grown in  $F_4$  bred true or almost true for early maturity, and some of the midseason selections also bred true or nearly true in  $F_4$ . True-breeding strains from 7 to 10 days earlier than the early parent (Niro Vialone) were obtained in  $F_4$ . Some strains, however, continued to segregate in  $F_4$  for early and midseason maturity.

The data herein presented on the inheritance of earliness in rice in the  $F_1$ ,  $F_2$ ,  $F_3$ , and  $F_4$  generations indicate that, while earliness probably is inherited in the same manner as other quantitative characters, the  $F_2$  population studied could not be placed with any degree of certainty in any of the simple or modified Mendelian ratios. Transgressive inheritance occurred and there was a heaping up of the plants in the intermediate position in  $F_2$  populations. In the  $F_3$  progeny one family bred almost true for early maturity and one almost true for late maturity. Three other families segregated in a ratio of approximately 3 early to 1 late plant, and one family produced plants in a distinct ratio of 1 early to 2 midseason to 1 late. Other families did not appear, however, to segregate in any definite ratio, for the segregating plants were distributed somewhat like the  $F_2$  plants. The data presented indicate that two or more genetic factors are involved in the production of earliness in the rices studied.

#### LENGTH OF GROWING PERIOD

The average number of days from the date of first irrigation to maturity for the Niro Vialone and Caloro varieties during the six-year period from 1918 to 1923, inclusive, was 134 and 154 days, respectively. Niro Vialone therefore is normally about 20 days earlier than Caloro.

The mean number of days to maturity of the parent plants and the  $F_2$  progeny, with the standard deviation and coefficient of variation of the  $F_2$ , are given in Table 6. It is observed that the parent varieties were mature in 129 and 143 days, respectively, from the date of the first irrigation. The fact that the parent varieties required less time in 1924 to reach maturity than the average number of days given for 1923 probably was due to the fact that they were started in a lighter soil which was warmer and more favorable for germination and early growth. It should also be remembered that the parent plants were transplanted in 1924, and this, too, may have induced earlier maturity. Climatic factors from year to year also have a marked influence upon the date of maturity of a given variety. In 1924 rice ripened rapidly, due apparently to the favorable temperatures and low humidity.

TABLE 6.—Mean length of growing season in days, standard deviation, and coefficient of variation for days to maturity of plants of  $F_2$  hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924

Material	Number of plants	Mean number of days to maturity	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	207	135.64±0.413	8.786±0.292	6.477±0.215
Niro Vialone.....	32	129		
Caloro.....	34	143		

The mean length of the growing period of the  $F_2$  progeny was 135.6 days. This is almost exactly the average of the parent varieties, which was 136 days. The standard deviation of the  $F_2$  progeny shows considerable variability with respect to length of growing period, but this is to be expected in a heterozygous population.

The data on the mean length of the growing period of the  $F_3$  plants and parent varieties are shown in Table 7. The mean length of this period for the  $F_3$  plants was intermediate between that of the parents, but somewhat nearer the late parent, Caloro. The standard deviation indicates that the  $F_3$  plants were much more variable in respect to

*SEEDING TO FIRST SPIKELET EMERGENCE, DAYS*

	70.5	73.5	76.5	79.5	82.5	85.5	88.5	91.5	94.5	97.5	100.5	103.5	TOTALS
<i>YIELD PER PLANT, GRAMS</i>													
3-5								1					1
5-7													0
7-9			1		1								2
9-11						1			1	1			3
11-13			1			1	1						3
13-15			1	2									3
15-17		1	2	2	2	2	3	1	2	1			16
17-19	2	2	3	2	1	1	2			1			14
19-21			2	2	2	5	4	3	1	2			21
21-23		2	1	2	4	1	1			3	2		16
23-25	1		2		3	5	4	1	2	1	1		20
25-27			2	2	4	5	2		1		2		18
27-29			2	2		3	3			2	1		13
29-31				3	3	1	3	1			1		12
31-33				1	1	5	4	1	1			1	14
33-35					2	5	2	2	1				12
35-37				1	2	6	2						11
37-39				1	1	1				1			4
39-41						3	1	1					5
41-43													0
43-45						1							1
45-47													0
47-49													0
49-51					1								1
TOTALS	3	5	17	20	27	45	33	11	9	12	7	1	190

FIG. 4.—Correlation table for 190  $F_2$  rice plants of the cross Niro Vialone×Caloro grown at Biggs, Calif., in 1924. Total yield of plant in grams, subject. Number of days from first irrigation to first spikelet emergence, relative.  $R_{xy}=0.0931\pm0.049$

date of maturity than were the parents and that the Caloro parent was much less variable than was the Niro Vialone parent.

TABLE 7.—Mean length of growing season in days, standard deviation, and coefficient of variation for days to maturity of plants of  $F_3$  hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925

Material	Number of plants	Mean number of days to maturity	Standard deviation	Coefficient of variation
$F_3$ hybrid.....	470	139.13±0.212	6.823±0.150	4.904±0.108
Niro Vialone.....	26	128.69±.317	2.398±.224	1.863±.174
Caloro.....	32	146.00±.067	.559±.047	.383±.032

In connection with date of maturity it is of interest to know whether there is a correlation between date of first spikelet emergence, or flowering, and plant yield. In Figure 4 is given a correlation table for 190  $F_2$  rice plants from the cross Niro Vialone  $\times$  Caloro. Total yield per plant in grams is subject. Number of days to first spikelet emergence in 1924 is relative. In Figure 4,  $R_{xy} = 0.0931 \pm 0.049$ , which shows that there apparently is no correlation between the yield of plant and number of days to first spikelet emergence. The fact

	NUMBER OF CULMS PER PLANT																									TOTALS
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25				
3-5				1																				1		
5-7																								0		
7-9			1			1																		2		
9-11	1		1	1																				3		
11-13		1		1	1																			3		
13-15				2	1																			3		
15-17		3	4	3	2	1	1		1	1														16		
17-19		4	1	1	5	1		2																14		
19-21		1	1	6	2	1	1	6	1		1	1												21		
21-23		1	2	2	2	4	4			1														16		
23-25				2	1	5	5	5	2															20		
25-27				2	1	4	5	3		1	2													18		
27-29			1		1	1		2	3	2	3													13		
29-31						2	2	2		1	2	1		1		1								12		
31-33						2	2	1	4	2	2	1												14		
33-35					1		1	2	3	3			1		1									12		
35-37					1	1	1	1	2	1	1	2		1										11		
37-39							2			1	1													4		
39-41										1			1	2		1								5		
41-43																								0		
43-45																						1	1			
45-47																								0		
47-49																								0		
49-51													1											1		
TOTALS	1	10	11	21	18	23	24	24	16	14	12	6	2	4	1	2	0	0	0	0	0	0	1	190		

FIG. 5.—Correlation table for 190  $F_2$  rice plants of the cross Niro Vialone  $\times$  Caloro grown at Biggs, Calif., in 1924. Total yield of plant in grams, subject. Number of culms per plant, relative.  $R_{xy} = 0.670 \pm 0.027$

that these two characters are not correlated indicates that it may be possible to secure by recombination of characters an early-maturing, high-yielding rice.

There was a positive and significant correlation between the yield per plant and the number of culms per plant,  $R_{xy} = 0.670 \pm 0.027$ , as is shown in Figure 5. There was no correlation, however, between the yield per plant and the length of panicles,  $R_{xy} = -0.080 \pm 0.049$ . Likewise, the yield per plant and height of plant were not correlated,  $R_{xy} = 0.0601 \pm 0.049$ .

## INHERITANCE OF YIELD

Yield undoubtedly is affected by the morphological and physiological characters of the plant as well as by the environmental complex. Engledow and Wadham (3) state:

Cereal yield is controlled by a great number of factors which are themselves complex and imperfectly understood. In approaching the "yield problem" it is convenient to arrange these in broad categories which may thus be designated: (i) Soil, (ii) Climate, (iii) Agricultural practice, (iv) Disease and Damage, (v) Botanical variety or form.

The yields of  $F_2$  plants and of the parents were obtained in the Niro Vialone  $\times$  Caloro cross. The  $F_2$  and parent plants, as stated before, were grown in rows spaced 3 feet apart, and the plants were spaced approximately 6 inches apart in the rows. The panicles were cut from the standing plants at maturity, and those of individual plants were placed in large manila envelopes. The envelopes were stored in the laboratory for about two months before the panicles were hand threshed and the grain weighed.

TABLE 8.—Mean yield, standard deviation, and coefficient of variation for yield of plant of  $F_2$  hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924

Material	Number of plants	Mean yield (grams)	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	190	25.16 $\pm$ 0.377	7.708 $\pm$ 0.267	30.636 $\pm$ 1.155
Niro Vialone.....	30	19.87 $\pm$ .603	4.894 $\pm$ .426	24.630 $\pm$ 2.271
Caloro.....	32	24.06 $\pm$ .652	5.465 $\pm$ .461	22.714 $\pm$ 2.011

The mean yield, standard deviation, and coefficient of variation for the  $F_2$  plants and the parent varieties are given in Table 8. It is seen that the mean plant yield of the  $F_2$  progeny is greater than that of the parent varieties. The standard deviation and coefficient of variation show that the  $F_2$  progeny were more variable than the parents. Yields of 470  $F_3$  plants from nine families were obtained in 1925. The plants were grown in rows spaced 2 feet apart, and the plants were spaced about 6 inches apart in the rows. Only plants that were spaced approximately 6 inches apart were harvested for a study of the agronomic characters. The data obtained on yield for the  $F_3$  and parent plants are given in Table 9.

TABLE 9.—Mean yield, standard deviation, and coefficient of variation for yield of plant of  $F_3$  progeny and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925

Material	Number of plants	Mean yield (grams)	Standard deviation	Coefficient of variation
$F_3$ hybrid.....	470	25.76 $\pm$ 0.242	7.780 $\pm$ 0.171	30.202 $\pm$ 0.722
Niro Vialone.....	26	45.46 $\pm$ 1.076	8.131 $\pm$ .760	17.886 $\pm$ 1.726
Caloro.....	32	45.73 $\pm$ .892	7.484 $\pm$ .631	16.366 $\pm$ 1.416

The  $F_3$  plants included early, midseason, and late forms, and all nine  $F_3$  families from which the 470 plants were harvested were segregating for earliness. Many of the late and some of the midseason

F<sub>3</sub> plants did not set seed and ripen well as a result of unfavorable blooming and ripening weather. Yields of the F<sub>3</sub> plants were quite variable, therefore, and lower than would be expected under more favorable climatic conditions.

Inspection of Table 9 shows that the mean yields of the parent varieties for the limited number of plants grown were not significantly different. The mean yield of the F<sub>3</sub> plants, however, was significantly lower than that of the parents. Using the coefficient of variation as a measure of variability, it is noted that the F<sub>3</sub> plants were much more variable than either parent, Caloro being slightly less variable than Niro Vialone.

#### INHERITANCE OF PLANT HEIGHT

Height of plant is an important character in rice, because the rice crop in the United States is harvested with grain binders. It is an essential requirement, therefore, that commercial varieties grow tall enough to bind well. In this study the height of the F<sub>2</sub> generation and of the parent varieties was measured at maturity. Height was measured from the base of the culms to the tip of the glume of the uppermost spikelet.

The mean height, standard deviation, and coefficient of variation for height of plant in F<sub>2</sub> progeny and for the parent varieties is shown in Table 10. The parent varieties are approximately of the same height and show no significant difference in the amount of variation. However, the Caloro parent was a little more variable than the Niro Vialone parent, as is shown by the coefficient of variation. The mean height of the F<sub>2</sub> plants is greater than that of the parents. The difference in mean height of the F<sub>2</sub> plants and the parents is probably significant in each case, for it is more than six times the probable error. The F<sub>2</sub> plants also were considerably more variable with respect to height than either parent.

TABLE 10.—*Mean height, standard deviation, and coefficient of variation for height of plant of F<sub>2</sub> hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean height (inches)	Standard deviation	Coefficient of variation
F <sub>2</sub> hybrid.....	207	36.69±0.142	3.019±0.100	8.228±0.273
Niro Vialone.....	32	35.44±.143	1.197±.101	3.378±.285
Caloro.....	34	35.50±.144	1.243±.102	3.501±.286

The data on the height of F<sub>3</sub> plants are shown in Table 11. The F<sub>3</sub> progeny are significantly shorter than either parent, but tall enough to bind well. The standard deviation and coefficient of variability show that the F<sub>3</sub> plants were much more variable in height than was either parent. This indicates that tall or short strains may be obtained if desired. The fact that the F<sub>2</sub> hybrid plants were taller than the parents and the F<sub>3</sub> plants shorter than the parents may indicate that heterosis influenced the height of the F<sub>2</sub> plants.

TABLE 11.—*Mean height, standard deviation, and coefficient of variation for height of plant of  $F_2$  hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925*

Material	Number of plants	Mean height (inches)	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	470	34.57 $\pm$ 0.063	2.903 $\pm$ 0.066	8.658 $\pm$ 0.190
Niro Vialone.....	26	42.77 $\pm$ .128	1.971 $\pm$ .091	2.270 $\pm$ .212
Caloro.....	32	38.97 $\pm$ .236	1.976 $\pm$ .167	5.071 $\pm$ .428

## INHERITANCE OF NUMBER OF CULMS

Usually those varieties of rice which have a large number of culms per plant produce higher yields per plant and, under certain conditions, higher yields per acre than those varieties which do not tiller freely. Because of this relationship between yield and number of culms it is important to keep the stooling ability of plants in mind in a study of this nature. The number of culms in the  $F_2$  and parent plants of the cross Niro Vialone  $\times$  Caloro were recorded. The mean number of culms, standard deviation, and coefficient of variation for number of culms of the  $F_2$  and the parent plants are given in Table 12.

TABLE 12.—*Mean number of culms, standard deviation, and coefficient of variation for number of culms per plant of  $F_2$  hybrids and for Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924*

Material	Number of plants	Mean number of culms	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	207	10.48 $\pm$ 0.167	3.555 $\pm$ 0.118	33.922 $\pm$ 1.247
Niro Vialone.....	32	6.19 $\pm$ .306	2.567 $\pm$ .216	41.470 $\pm$ 4.053
Caloro.....	34	14.44 $\pm$ .514	4.440 $\pm$ .363	30.748 $\pm$ 2.742

In Table 12 it is interesting to note that the mean number of culms per plant for the Niro Vialone parent was 6.19, for the Caloro parent 14.44, and for the  $F_2$  plants 10.48 culms per plant. The mean number of culms per plant of the  $F_2$  progeny was almost exactly the average of the parents. Greater variation would be expected in the  $F_2$  plants than in either parent, but the coefficient of variation shows that the Niro Vialone parent was more variable, while the Caloro parent was less variable than the  $F_2$  progeny.

Data were obtained on the mean number of culms of 470  $F_3$  plants and the parent varieties, and these data are given in Table 13. The mean number of culms of the  $F_3$  plants was significantly less than that of either parent. The coefficients of variation indicate that the  $F_3$  plants were not significantly more variable with respect to number of culms than were the parents. The low mean yield obtained for the  $F_3$  plants very probably is correlated with the small mean number of culms per plant.



TABLE 13.—Mean number of culms, standard deviation, and coefficient of variation for number of culms per plant of  $F_2$  hybrids and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925

Material	Number of plants	Mean number of culms	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	470	7.23±0.087	2.786±0.062	38.672±0.970
Niro Vialone.....	26	10.73±.539	4.072±.381	37.950±4.028
Caloro.....	32	13.03±.593	4.972±.419	38.158±3.656

## INHERITANCE OF PANICLE LENGTH

The length of panicle in rice may or may not be closely associated with yield per plant. The length of the panicles of the  $F_2$  plants and of the parents was recorded in this study. The panicles were measured in the laboratory, from the base of the panicle to the glume tips of the uppermost spikelet. The average length of all panicles on a plant was used as the panicle length for that particular plant. The mean panicle length for the parents and  $F_2$  progeny, with the standard deviations and coefficients of variation, are shown in Table 14.

TABLE 14.—Mean length of panicle, standard deviation, and coefficient of variation for length of panicle of plants of  $F_2$  hybrid and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1924

Material	Number of plants	Mean length of panicle (inches)	Standard deviation	Coefficient of variation
$F_2$ hybrid.....	207	7.17±0.028	0.601±0.020	8.382±0.278
Niro Vialone.....	32	7.26±.030	.250±.021	3.444±.290
Caloro.....	34	6.51±.049	.427±.035	6.559±.536

Table 14 shows that the mean length of panicle of the early parent, Niro Vialone, was 18.45 cm. (7.26 in.), of the  $F_2$  progeny 18.20 cm. (7.17 in.), and of the midseason parent, Caloro, 16.53 cm. (6.51 in.), respectively. In length of panicle the  $F_2$  progeny are intermediate between the parents, but nearer the early parent, which had the longest panicles. In this connection it may be of interest to note that while the mean length of panicle for the early Niro Vialone parent was distinctly longer than that of the midseason Caloro parent, the latter produced a mean yield per plant of 24.06 gm., while the mean yield for the Niro Vialone plant was only 19.87 gm. The main reason for this is that the Caloro parent produced an average of about 14 culms per plant and Niro Vialone only about 6 culms per plant.

The coefficients of variation for length of panicle show that the  $F_2$  progeny was more variable than either parent, the Niro Vialone parent being least variable with respect to panicle length. The greater variation of the Caloro parent no doubt is due in part to the tendency of midseason and late-maturing rice varieties to send out late tillers which often produce only short panicles. This formation of late culms is not nearly so common in early-maturing varieties of rice, which usually produce few culms bearing panicles of a comparatively uniform length.

Data on panicle length of the  $F_3$  and parent plants are given in Table 15. It is observed that the panicles of the  $F_3$  plants were significantly shorter than those of Niro Vialone and of about the same length as those of Caloro. The coefficients of variation indicate that the panicles of the  $F_3$  plants were more variable in length than those of either parent.

TABLE 15.—Mean length of panicle, standard deviation, and coefficient of variation for length of panicle of plants of  $F_3$  hybrid and for the Niro Vialone and Caloro parent varieties of rice grown at Biggs, Calif., in 1925

Material	Number of plants	Mean length of panicle (inches)	Standard deviation	Coefficient of variation
$F_3$ hybrid.....	470	7.29±0.018	0.593±0.013	8.134±0.179
Niro Vialone.....	26	7.52±.034	.259±.024	3.444±.322
Caloro.....	32	7.34±.041	.341±.029	4.646±.392

### INHERITANCE OF SHAPE OF NECK

The elongated internode which bears the inflorescence of grasses is called the peduncle. That portion of the peduncle immediately below the panicle often is called the neck. In most rice varieties the neck is straight. Varieties with sinuous necks, however, are not uncommon.

In the Niro Vialone variety the neck is sinuous, while in Caloro the neck is straight. The  $F_1$  hybrid from the cross Niro Vialone × Caloro had sinuous necks. In the  $F_2$  progeny segregation occurred, producing 197 sinuous-necked plants to 10 plants with straight necks. This segregation suggests that duplicate factors are responsible for the sinuous-neck character, as is shown in Table 16; producing approximately a 15 : 1 ratio of sinuous-necked and straight-necked plants. The deviation from the calculated numbers in the 15 : 1 ratio is small and apparently not significant.

TABLE 16.—Segregation of  $F_2$  hybrids in the Niro Vialone × Caloro rice cross for sinuous-necked and straight-necked plants at Biggs, Calif., in 1924

Shape of neck	Number of plants		Deviation from 15 : 1 ratio	Probable error <sup>a</sup>
	Observed	Calculated		
Sinuous.....	197	194.06	+2.94	±2.35
Straight.....	10	12.94	-2.94	2.35

<sup>a</sup> The probable errors for numbers of individuals given in this paper were obtained from tables of probable errors of Mendelian ratios prepared by the department of plant breeding, Cornell University, Ithaca, N. Y.

If the sinuous-neck character is due to dominant duplicate factors, as the  $F_2$  data indicate, then in the  $F_3$  generation there should be families that breed true for sinuous necks, others that breed true for straight necks, and still others that segregate in ratios of approximately either 3 or 15 sinuous-necked plants to 1 straight-necked.

Forty-seven  $F_3$  families were grown in 1925, and the segregation of the heterozygous families is given in Table 17. Inspection of Table 17 shows that 14 families segregated in the ratio of 3 sinuous-necked

plants to 1 straight-necked, and 16 families segregated in the ratio of 15 sinuous-necked plants to 1 straight-necked. Four families, however, segregated in the ratio of about 9 sinuous-necked plants to 7 straight-necked. In 5 other families the ratio was about 1 sinuous-necked plant to 2 straight-necked ones. Eight  $F_3$  families bred true for the sinuous-neck character.

TABLE 17.—Segregation of 39  $F_3$  families of the Niro Vialone  $\times$  Caloro rice cross for sinuous necks and straight necks at Biggs, Calif., in 1925

[Symbols for neck shape: S=sinuous, St.=straight]

F <sub>2</sub> family No. and ratio	Shape of neck in F <sub>2</sub>	Number of F <sub>3</sub> plants with necks—		Calculated on ratio named	Deviation	Probable error ±
		Sinuous	Straight			
Calculated on 3.1 ratio:						
221A1.....	S	91	25	29.00	-4.00	3.15
14.....	S	106	18	31.00	-13.00	3.25
29.....	S	88	19	26.75	-7.75	3.02
37.....	S	85	32	29.25	+2.75	3.16
38.....	S	73	36	27.25	+8.75	3.05
42.....	S	53	26	19.75	+6.25	2.60
43.....	S	73	17	22.50	-5.50	2.77
54.....	S	70	18	22.00	-4.00	2.74
59.....	S	88	45	33.25	+11.75	3.37
80.....	S	65	11	19.00	-8.00	2.55
140.....	St.	63	26	22.25	+3.75	2.76
165.....	S	66	13	19.75	-6.75	2.60
170.....	S	105	25	32.50	-7.50	3.33
172.....	St.	100	31	32.75	-1.75	3.34
Total.....		1,126	342	367.00	-25.00	11.17
Calculated on 15:1 ratio:						
221A4.....	St	97	9	6.625	+2.375	1.68
22.....	S	133	3	8.500	-5.50	1.90
23.....	S	103	11	7.125	+3.875	1.74
26.....	S	106	14	7.500	+6.50	1.79
28.....	S	125	3	8.000	-5.00	1.85
35.....	S	83	11	5.875	+5.125	1.58
41.....	S	111	3	7.125	-4.125	1.74
61.....	S	135	2	8.5625	-6.5625	1.91
69.....	S	130	4	8.375	-4.375	1.89
110.....	S	110	4	7.125	-3.125	1.74
114.....	S	112	2	7.125	-5.125	1.74
133.....	S	93	1	5.875	-4.875	1.58
154.....	St.	79	5	5.250	-2.50	1.50
167.....	S	96	5	6.3125	-1.3125	1.64
168.....	S	128	2	8.125	-6.125	1.86
190.....	S	110	3	7.0625	-4.0625	1.74
Total.....		1,751	82	114.5625	-32.5625	6.97
Calculated on 9:7 ratio:						
221A12.....	St.	52	47	43.3125	+3.6875	3.33
51.....	S	62	40	44.6250	-4.6250	3.38
95.....	S	77	58	59.0625	-1.0625	3.89
179.....	S	62	47	47.6875	-6.875	3.49
Total.....		253	192	194.6875	-2.6875	7.06
Calculated on 1:2 ratio:						
221A13.....	S	43	61	34.67	+8.33	-----
15.....	S	49	62	37.00	+12.00	-----
63.....	S	31	78	36.33	-5.33	-----
83.....	S	30	97	42.33	-12.33	-----
173.....	S	37	83	40.00	-3.00	-----
Total.....		190	381	190.33	-.33	7.60
221A30.....	S	96	-----	-----	-----	-----
45.....	S	111	-----	-----	-----	-----
50.....	S	102	-----	-----	-----	-----
60.....	S	69	-----	-----	-----	-----
81.....	S	115	-----	-----	-----	-----
88.....	S	70	-----	-----	-----	-----
163.....	S	106	-----	-----	-----	-----
174.....	S	126	-----	-----	-----	-----
Total.....		795	-----	-----	-----	-----

This neck character is rather difficult to classify correctly. Both senses, sight and touch, can be used to advantage in classifying the plants, and even then errors are very likely to occur. Climatic conditions may prevent the plants from shooting promptly, and often the leaf sheaths are so firmly clasped about the panicles that complete emergence of the panicle is prevented, and the necks in such cases often are bent and may be twisted, especially if climatic conditions are unfavorable during the shooting period, as they were in 1925 at Biggs.

It is probable that the four  $F_3$  families segregating in the ratio of about 9 : 7 actually belong with those families that segregated in the ratio of 3 sinuous-necked plants to 1 straight-necked. The five families that segregated in the ratio of about 1 sinuous-necked plant to 2 straight-necked are difficult to dispose of and indicate that probably other disturbing factors are affecting the segregation for this character.

On the basis of  $F_3$  breeding behavior, the  $F_2$  ratio is changed from 197 sinuous-necked plants to 10 straight-necked to 202 sinuous-necked plants to 5 straight-necked. The deviation from calculated 15 : 1 ratio is  $7.94 \pm 2.35$  plants. The deviation, therefore, is about 3.4 times its probable error and may be significant.

The fact, however, that 30  $F_3$  families produced expected ratios and 8 families bred true for sinuous necks indicates quite conclusively that at least two factors are involved in the production of the sinuous neck in the variety used in this study.

#### SUMMARY

This study was made on breeding material grown in an effort to obtain an early-maturing, high-yielding rice of good quality.

The Niro Vialone and Caloro varieties were used as parents in this cross because they appeared to be very good material for combining a study of the inheritance of contrasting characters with the possibility of obtaining in one variety early maturity, high yield, and good quality.

The cross was made and the material grown at Biggs, Calif.

$F_1$  hybrids from various rice crosses may be earlier than the early parent, later than the late parent, nearer the early or late parent, and the parents may be so different that the  $F_1$  hybrids are sterile. The  $F_1$  plant of the cross Niro Vialone  $\times$  Caloro was nearer the early parent.

The segregation of  $F_2$  progeny, from the crosses Niro  $\times$  Caloro and Eureka  $\times$  Caloro, for date of spikelet exertion, shows that most of the plants were intermediate. In each case, however, transgressive inheritance occurred.

In the  $F_3$  families of the cross Niro Vialone  $\times$  Caloro there was one family that bred almost true for earliness, one family that was late maturing, three families that segregated in a ratio of about 3 early-maturing plants to 1 late, one family that gave a distinct ratio of 1 early to 2 midseason to 1 late, and other families that gave distributions somewhat like the  $F_2$  hybrids.

Some of the early selections made from the  $F_3$  progeny bred true in  $F_4$ . True breeding types were obtained that are from 7 to 10 days earlier than the early parent and are promising in yield and quality.

The  $F_2$  hybrids were intermediate in date of maturity. Early and late-maturing  $F_3$  selections were grown in 1925. Strains considerably earlier than the early parent were isolated.

There was apparently no correlation between the yield of  $F_2$  plants and date of first spikelet exertion. This indicates that high yield per plant and earliness may be combined in the same variety. Yields per plant and number of culms were definitely correlated, but length of panicles and height of plant were not correlated with yield per plant.

Yield is undoubtedly due to multiple factors. The  $F_2$  hybrids exceeded the parents in yield and variability. The yields of the  $F_3$  progeny were less than those of either parent, but were much more variable.

Tallness of plant appeared to be partially dominant in  $F_2$ , due probably to heterosis, the  $F_3$  selections being significantly shorter than either parent.

In number of culms per plant the  $F_2$  hybrids were almost exactly intermediate between those of the parents. The  $F_3$  selections had fewer culms per plant than either parent, but were equally variable.

In length of panicle the  $F_2$  hybrids were intermediate, but nearer the early parent, which has the longer panicles. The  $F_3$  selections had shorter panicles than either parent. Variability was greater.

Sinuous necks were dominant to straight necks in  $F_1$ , and the  $F_2$  and  $F_3$  segregations suggest that at least duplicate genetic factors were probably involved.

#### LITERATURE CITED

- (1) AKEMINE, M.  
1914. ÜBER DAS BLÜHEN DES REISES UND EINIGE SICH DARAN ANKNÜPFENDE ERSCHEINUNGEN. *Ztschr. Pflanzenzücht.* 2:339-375, illus.
- (2) BHIDE, R. K.  
1926. INHERITANCE AND CORRELATION OF CERTAIN CHARACTERS IN RICE CROSSES. *Poona Agr. Col. Mag.* 18:76-85.
- (3) ENGLEADOW, F. L., and WADHAM, S. M.  
1923. INVESTIGATIONS ON YIELD IN THE CEREALS. I. *Jour. Agr. Sci. [England]* 13:390-439, illus.
- (4) EVANS, G.  
1922. REPORT OF THE AGRICULTURAL DEPARTMENT, BENGAL, FOR 1921-22. *Bengal Dept. Agr., Rpt. 1921/22*, 138 p.
- (5) HOSHINO, Y.  
1915. ON THE INHERITANCE OF THE FLOWERING TIME IN PEAS AND RICE. *Jour. Col. Agr., Tohoku Imp. Univ.* 6:229-288, illus.
- (6) IKENO, S.  
1918. A TEXT-BOOK ON GENETICS. Ed. 3, 230 p., illus. Tokyo. [In Japanese. Abstract in *Bot. Abs.* 2:114-115. 1919.]
- (7) NOMURA, M., and YAMAZAKI, R.  
1925. A STUDY OF THE INHERITANCE OF THE SHOOTING TIME IN RICE-PLANT. *Japan. Jour. Genetics* 3:112-130. [In Japanese. Abstract in *Japan. Jour. Bot.* 3:(18). 1926.]



# ILLUSTRATIONS OF THE APPLICATION OF A CRITERION OF THE DEVIATION OF AN OBSERVED FROM A RANDOM DISTRIBUTION TO THE PROBLEM OF SEEDLING STAND IN SEA-ISLAND, EGYPTIAN, AND UPLAND COTTON<sup>1</sup>

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## INTRODUCTION

In practical agriculture an effort should be made to obtain in each hill planted an adequate number of seedlings to produce a full stand without waste of seed. Hills which contain no seedlings represent (in a field with proper spacing of hills) a waste of potentially productive land. Hills which contain a larger number of seedlings than is required to produce a full stand of suitable plants represent not merely a waste of seed in planting but result in too dense a stand in some places and necessitate the expense of thinning.

That the condition of the field as well as the quality of the seed influences the crop stand is well known to all agriculturists. Certain fields may, under the conditions of a particular season, produce stands so poor as to necessitate replanting, while other fields produce stands that are highly satisfactory. The physical causes of such differences may be clearly evident in a particular season, or they may be obscure.

In a single field the stand secured is often irregular. From inspection alone it may prove quite impossible to determine whether the observed irregularities are caused by diversities of the soil or other local influences or whether they are merely the result of the random distribution of seedlings when the stand as a whole is below the desired optimum.

It is impossible to hope for an entire absence of variation in seedling stand. The stand which most ideally meets practical needs would seem to be that in which the seedlings are distributed as uniformly as possible over the field. This will involve distribution in a random manner about a mean number such that practically no hills without seedlings and practically no hills with excessively large numbers of seedlings occur.

In work on disease resistance, in critical comparisons of the physiological characteristics of varieties, or in refined agronomic experiments of other kinds, it may sometimes become necessary to determine not merely the percentage germination which may be obtained in the field under given conditions but also the nature of the distribution of the stand over the field.

The specific problem here considered—that of the distribution of number of seedlings per hill—is highly complicated. Its final solution involves considerations of the germination rate, as such, of seeds exposed to field conditions, and the relationship of this rate

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<sup>1</sup> Received for publication Jan. 3, 1928; issued June, 1928.

to the rate determined under laboratory conditions, of the physical factors of the soil and the physiological and morphological characteristics of the seedlings which may determine the emergence of the germinated seed from the soil, and of the relative susceptibility of different varieties to damping-off diseases. It is one phase of the general problem of field heterogeneity, which has been considered in a number of investigations for the Bureau of Plant Industry.

It is impossible to attack all these varied problems in one investigation, but it is reasonable to expect that the establishment of the existence of underlying causes of observable irregularities would necessitate as a first step the application of some criterion to test the significance of the deviation of the observed germination frequencies from a purely random distribution. It is the purpose of this paper to illustrate the use of a criterion applicable in certain cases, by concrete examples drawn from cultures of Egyptian, sea-island, and upland cottons as grown under irrigation at the United States Field Station of the United States Department of Agriculture and the United States Office of Indian Affairs at Sacaton, Ariz.<sup>2</sup> In this paper attention is limited to a comparison of the actual frequency distribution of the number of seedlings per hill with the theoretical distribution which should arise if frequencies were determined solely by chance.

#### METHOD OF DETERMINING THEORETICAL DISTRIBUTION OF NUMBER OF SEEDLINGS PER HILL

In determining the theoretical distribution of number of seedlings per hill the procedure is as follows:

Let  $s$  be the number of seeds planted per hill in  $N$  hills,  $g$  the number germinating per hill, and  $f$  the number failing to germinate per hill (or to survive to any stage desired). Then  $s = g + f$ .

Let  $\bar{g}$  and  $\bar{f}$  be the mean number of seeds germinating and failing to germinate, respectively, per hill for the tract as a whole. Then the chances, for the field as a whole, of germination,  $p$ , and that of failure,  $q$ , are defined as follows:

$$p = \frac{\bar{g}}{s} = \frac{\Sigma(g)}{Ns}$$

and

$$q = \frac{\bar{f}}{s} = \frac{\Sigma(f)}{Ns} = 1 - p$$

Now  $p + q = 1$ , and the distribution (assumed to be due solely to chance) of the relative frequencies when the number of seeds planted per hill is  $s$  (and is constant for the experimental tract under investigation) will be represented by the terms of the expression  $(p + q)^s$ , while the theoretical frequency for a given tract will be represented by the corresponding terms of  $N(p + q)^s$ , where  $N$  is the number of hills.

The significance of the divergence of the frequency distribution actually formed from the theoretical frequency distribution of number of seedlings per hill thus determined may be tested by means of Pearson's  $\chi^2$  criterion of goodness of fit,<sup>3</sup> employing Elderton's

<sup>2</sup> The writers thank C. J. King, superintendent of the station, most heartily for facilities provided and for his continued interest in the work.

<sup>3</sup> PEARSON, K. ON THE CRITERION THAT A GIVEN SYSTEM OF DEVIATIONS FROM THE PROBABLE IN THE CASE OF A CORRELATED SYSTEM OF VARIABLES IS SUCH THAT IT CAN BE REASONABLY SUPPOSED TO HAVE ARISEN FROM RANDOM SAMPLING. Phil. Mag. 50: 157-175. 1900.



tables <sup>4</sup> of the values of  $P$  to translate the values of  $\chi^2$  into terms of probability.

The simplest method of calculation may be illustrated by the entries for Pima cotton shown in Table 3.

The frequencies of germination of 0 to 6 seedlings per hill, respectively, in the 1,440 hills lead to  $\Sigma(g)=2,844$ ,  $\bar{g}=1.975000$ ,

$$p = \frac{\Sigma(g)}{6N} = \frac{\bar{g}}{6} = 0.329167, \quad 1-p = 0.670833$$

Table 1 shows the most convenient process of computing  $N(p+q)^6$ , which leads to the theoretical distribution in the final column.

TABLE 1.—*Calculation of theoretical distribution of number of seedlings per hill in Pima Egyptian cotton (experiment 3/22) as grown at Sacaton, Ariz., in 1922*

Powers of $p$	Powers of $q$	Products of powers of $p$ and $q$	Coefficients	$(p+q)^6$	$N(p+q)^6$
-----	$q^6=0.091135$	$q^6=0.091135$	1	0.091135	131.2348
$p=0.329167$	$q^5=.135854$	$pq^5=.044719$	6	.268312	386.3688
$p^2=.108351$	$q^4=.202515$	$p^2q^4=.021943$	15	.329141	473.9625
$p^3=.035666$	$q^3=.301886$	$p^3q^3=.010767$	20	.215339	310.0877
$p^4=.011740$	$q^2=.450017$	$p^4q^2=.005284$	15	.079255	114.1276
$p^5=.003864$	$q=.670833$	$p^5q=.002592$	6	.015554	22.3980
$p^6=.001272$	-----	$p^6=.001272$	1	.001272	1.8317
				1.000000	1,440.0000

A comparison of actual and theoretical frequencies in Table 2 leads to

$$S \left[ \frac{(o-c)^2}{c} \right] = \chi^2 = 4372.7698$$

The number of decimal places retained throughout is larger than is required in most cases.

TABLE 2.—*Comparison of observed (o) and calculated (c) number of seedlings per hill in Pima Egyptian cotton and calculation of  $\chi^2$*

Seedlings per hill	Observed number of hills (o)	Calculated number of hills (c)	Observed minus calculated (o-c)	$\frac{(o-c)^2}{c}$
0	517	131.23	+385.77	1,134.0279
1	196	386.37	-190.37	93.7980
2	179	473.96	-294.96	183.5628
3	168	310.09	-142.09	65.1087
4	181	114.13	+66.87	39.1799
5	132	22.40	+109.60	536.2571
6	67	1.83	+65.17	2,320.8354
Total...	1,440	1,440.01	-000.01	4,372.7698

## EXPERIMENTAL METHODS AND MATERIALS

The plantings were made by hand at the United States Field Station, Sacaton, Ariz., by careful workers experienced in experimental cotton culture. While the stand produced was low and the proportion of hills which failed to produce any seedlings was large, there was reason

<sup>4</sup> ELDERTON, W. P. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. *Biometrika* 1: 155-163. 1902.

to believe that the experimental technic could not have been improved.

Planting was standardized to six seeds per hill in all cases, in part because of the requirements of this phase of the investigations and in part because experience has shown that in the case of cotton grown under these conditions such a planting rate is necessary in order to obtain a reasonably full stand. This gives a possible range of 0 to 6 seedlings per hill. Seedling stand rather than germination rate serves as a basis for calculation, since the accurate determination of the number of seeds germinating under field conditions would be exceedingly difficult. Countings of number of seedlings were made when the plants had reached the stage of development at which thinning should be undertaken.

The data employed represent three types of cotton: Sea-island, Egyptian (Pima variety), and upland (Acala, Durango, Lone Star, and Meade varieties). The Pima Egyptian variety is also represented by a homozygous smooth-seeded form, while the Acala upland variety is also represented by a presumably mutant form known as okra-leaved Acala. These were grown in four experiments, the key numbers of which are here retained for convenience of reference.

#### EXPERIMENTAL DATA

Experiment 3/22 is a comparison of Pima Egyptian, Meade upland, and Acala upland cotton as grown in 1922. The organization of the experiment has been described in some detail in connection with a study of the relationship between the concentration of the soil solution and the physicochemical properties of the leaf-tissue fluids.<sup>5</sup> The properties of the soil and the physicochemical properties of the plant-tissue fluids have also been considered in connection with the problem of the nature of the regression of soil properties and crop characters in associated plots of the experimental field.<sup>6</sup> In brief, it represents plantings of 1,440 hills of each of these three varieties distributed over the north halves of the three adjacent plots E 3-1, E 3-2, and E 3-3, forming together a total area of 180 by 79.5 feet. The data are given in Table 3.

TABLE 3.—Seedling stand in Pima Egyptian and Meade and Acala upland cotton as grown at the United States Field Station (plots E 3-1 to E 3-3, experiment 3/22), Sacaton, Ariz., in 1922

Seedlings per hill	Number of hills		
	Pima Egyptian	Meade upland	Acala upland
0	517	645	412
1	196	279	236
2	179	181	186
3	168	174	242
4	181	98	200
5	132	44	125
6	67	19	39
Total...	1,440	1,440	1,440

<sup>5</sup> HARRIS, J. A. THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICO-CHEMICAL PROPERTIES OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. *Jour. Agr. Research* 32: 605-647, illus. 1928.

<sup>6</sup> HARRIS, J. A., CONNORS, I. L., ELDERS, A. T., and KIRK, L. E. ON THE REGRESSION OF SOIL PROPERTIES AND CROP CHARACTERS IN ASSOCIATED PLOTS OF AN EXPERIMENTAL FIELD. *Minn. Univ. Studies Biol. Sci.* 6: 351-371, illus. 1927.

Experiment 1/23 is a comparison of seedling stand in 1,440 hills each of Pima Egyptian and Lone Star upland cotton grown in 1923. The culture occupied an area of about 90 by 53 feet (omitting the area occupied by hybrid plants spaced between the two parent types and not here considered) on plots D 1-10 and D 1-11. The data are given in Table 4.

TABLE 4.—Seedling stand in Pima Egyptian and Lone Star upland cotton as grown at the United States Field Station (plots D 1-10 and D 1-11, experiment 1/23), Sacaton, Ariz., in 1923

Seedlings per hill	Number of hills	
	Pima Egyptian	Lone Star upland
0	850	879
1	171	165
2	104	112
3	107	113
4	97	89
5	73	64
6	38	18
Total---	1, 440	1, 440

Experiment 1/25 represents a comparison of the seedling stand in Pima Egyptian cotton, smooth-seeded Pima Egyptian cotton, Acala upland cotton, and okra-leaved Acala upland cotton. The plantings were distributed over two different plots. Plot D 2-3 comprised an area of 360 by 26.5 feet and carried 720 hills of each of these four varieties. The frequency distributions of number of seedlings per hill are presented in Table 5. Plot D 1-10 was used in part for this experiment. The area used was 200 by 26.5 feet and carried 400 hills each of the four varieties. The frequency distributions appear in Table 6.

TABLE 5.—Seedling stand in Pima Egyptian, smooth-seeded Pima, and Acala and okra-leaved Acala upland cotton as grown at the United States Field Station (plot D 2-3, experiment 1/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills			
	Pima Egyptian	Smooth-seeded Pima	Acala upland	Okra-leaved Acala
0	211	154	341	347
1	49	33	72	95
2	62	55	75	82
3	108	65	92	72
4	137	129	69	64
5	109	153	52	43
6	44	131	19	17
Total--	720	720	720	720

TABLE 6.—Seedling stand in Pima Egyptian, smooth-seeded Pima, and Acala and okra-leaved Acala upland cotton as grown at the United States Field Station (plot D 1-10, experiment 1/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills			
	Pima Egyptian	Smooth-seeded Pima	Acala upland	Okra-leaved Acala
0	51	56	121	116
1	34	22	85	59
2	37	34	58	62
3	56	41	54	72
4	80	82	36	46
5	82	87	35	31
6	60	78	11	14
Total--	400	400	400	400

Since the purpose in investigating the distribution of number of seedlings per hill is to test the influence of extremely localized conditions—physical or chemical properties of the soil, inability of a given group of seedlings to break through the overlying mass of soil, or irregularities of distribution of an infecting organism—on seedling stand, these two series (plots D 2-3 and D 1-10) may be combined in order to give frequency distributions of 1,120 hills each. Since such distributions may be formed by combining the entries of Tables 5 and 6, they need not be published here. The table of constants (Table 11) gives the results of calculations based on the combined material as well as on the two individual series.

Experiment 2/25 is a comparison of Pima Egyptian, sea-island, and Acala, Durango, Lone Star, and Meade upland cottons, made in 1925 on plot D 2-4, comprising an area of 360 by 26.5 feet. In this experiment, plantings of Pima Egyptian and sea-island cotton were repeated for each variety of upland cotton. Thus there are available 240 hills each of both Pima Egyptian and sea-island cotton for each of the upland varieties (Acala, Durango, Lone Star, and Meade), which were represented by 240 hills each. The data are given in Tables 7 to 10, each of which presents the frequency distribution of seedlings per hill in Pima Egyptian and sea-island cotton immediately associated with each of the four upland varieties considered.

TABLE 7.—Seedling stand in Pima Egyptian, sea-island, and Acala upland cotton as grown at the United States Field Station (plot D 2-4, experiment 2/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Acala upland
0	40	46	83
1	15	13	42
2	25	20	37
3	41	34	37
4	54	40	28
5	46	55	10
6	19	32	3
Total---	240	240	240

TABLE 8.—Seedling stand in *Pima Egyptian*, *sea-island*, and *Durango upland* cotton as grown at the United States Field Station (plot D 2-4, experiment 2/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Durango upland
0	47	68	63
1	16	14	17
2	30	15	15
3	36	23	28
4	48	34	42
5	49	50	50
6	14	36	25
Total...	240	240	240

TABLE 9.—Seedling stand in *Pima Egyptian*, *sea-island*, and *Lone Star upland* cotton as grown at the United States Field Station (plot D 2-4, experiment 2/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Lone Star upland
0	61	52	103
1	12	14	37
2	20	19	46
3	33	23	38
4	56	50	11
5	40	51	5
6	18	31	-----
Total...	240	240	240

TABLE 10.—Seedling stand in *Pima Egyptian*, *sea-island*, and *Meade upland* cotton as grown at the United States Field Station (plot D 2-4, experiment 2/25), Sacaton, Ariz., in 1925

Seedlings per hill	Number of hills		
	Pima Egyptian	Sea island	Meade upland
0	59	68	91
1	16	12	30
2	29	17	40
3	37	29	36
4	47	36	25
5	35	46	15
6	17	32	3
Total...	240	240	240

Since the *Pima Egyptian* and *sea-island* plants were grown in comparison with four upland varieties, the *Egyptian* and *sea-island* records may be treated in four individual groups each based on 240 hills. These may be designated *Pima with Meade*, *Pima with Lone Star*, *Pima with Acala*, and *Pima with Durango*. A similar notation applies to the *sea-island* variety. Since both of these varieties (*Pima Egyptian* and *sea island*) were distributed over the whole

experimental area, the four subseries may be combined to give a larger frequency distribution for both Pima Egyptian and sea-island cotton. These are designated merely as Pima Egyptian and sea island in Table 11.

TABLE 11.—*Values of  $\chi^2$  test for closeness of agreement of observed and theoretical frequency distributions of number of seedlings per hill in sea-island, Egyptian, and upland cotton*

Experiment and variety	Plot	Table No.	N	$\chi^2$
Experiment 3/22.				
Pima (Egyptian).....	E 3-1, E 3-2, E 3-3	3	1,440	4,372.8
Meade (upland).....	E 3-1, E 3-2, E 3-3	3	1,440	2,975.6
Acala (upland).....	E 3-1, E 3-2, E 3-3	3	1,440	10,601.5
Experiment 1/23:				
Pima (Egyptian).....	D 1-10, D 1-11	4	1,440	21,981.5
Lone Star (upland).....	D 1-10, D 1-11	4	1,440	12,072.4
Experiment 1/25:				
Pima (Egyptian).....	D 2-3	5	720	2,080.3
Smooth-seeded Pima (Egyptian).....	D 2-3	5	720	4,808.9
Acala (upland).....	D 2-3	5	720	2,687.9
Okra-leaved Acala (upland).....	D 2-3	5	720	3,080.5
Pima (Egyptian).....	D 1-10	6	400	1,148.5
Smooth-seeded Pima (Egyptian).....	D 1-10	6	400	2,104.7
Acala (upland).....	D 1-10	6	400	606.7
Okra-leaved Acala (upland).....	D 1-10	6	400	620.8
Pima (Egyptian).....	D 1-10, D 2-3	5, 6	1,120	3,523.8
Smooth-seeded Pima (Egyptian).....	D 1-10, D 2-3	5, 6	1,120	7,053.4
Acala (upland).....	D 1-10, D 2-3	5, 6	1,120	3,191.3
Okra-leaved Acala (upland).....	D 1-10, D 2-3	5, 6	1,120	3,227.6
Experiment 2/25:				
Pima (Egyptian) with Meade (upland).....	D 2-4	7-10	240	599.2
Pima (Egyptian) with Lone Star (upland).....	D 2-4	7-10	240	780.3
Pima (Egyptian) with Acala (upland).....	D 2-4	7-10	240	552.5
Pima (Egyptian) with Durango (upland).....	D 2-4	7-10	240	542.4
Pima (Egyptian).....	D 2-4	7-10	960	2,490.5
Sea island with Meade (upland).....	D 2-4	7-10	240	1,275.1
Sea island with Lone Star (upland).....	D 2-4	7-10	240	1,136.3
Sea island with Acala (upland).....	D 2-4	7-10	240	1,053.9
Sea island with Durango (upland).....	D 2-4	7-10	240	1,457.2
Sea island.....	D 2-4	7-10	960	4,933.2
Meade (upland).....	D 2-4	7-10	240	319.5
Lone Star (upland).....	D 2-4	7-10	240	132.1
Acala (upland).....	D 2-4	7-10	240	240.7
Durango (upland).....	D 2-4	7-10	240	1,033.2

## DISCUSSION OF DATA

The frequency distributions of number of seedlings per hill are given in Tables 3 to 10. Space precludes the presentation of the frequencies calculated on the assumption of chance distribution and the numerical values of the differences between the observed and the theoretical frequencies. These are, however, presented graphically in Figures 1, 2, and 3, where the actual percentage frequencies are shown by the height of the rectangles, while the computed percentage frequencies are shown by the height of the solid dots on the scale of ordinates.

In all of these series, which represent a considerable range of varieties and of soil and other conditions which may influence seedling stand, the more central classes of the frequency distributions show a deficiency of observed as compared with theoretical frequencies, whereas the lower and upper extremes show a material excess of observed as compared with theoretical frequencies. It is clear that environmental conditions inherent in the soil or differences in the capacities of the small groups of seedlings planted in the various hills

for lifting the soil mass are such as to favor high percentage germination or seedling survival in some places and low percentage germination or survival in others.

A comparison of the observed and theoretical distributions by means of Pearson's  $\chi^2$  test of goodness of fit<sup>7</sup> in Table 11 shows values of  $\chi^2$  which are very large in comparison with those given in

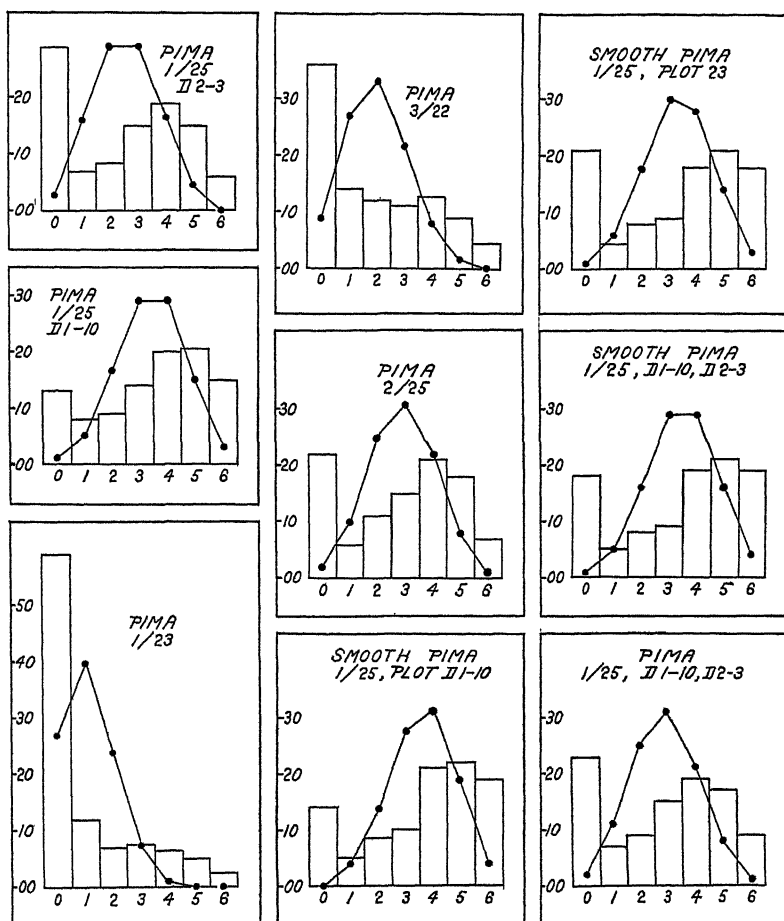


FIG. 1.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in Pima Egyptian cotton and smooth-seeded Pima Egyptian cotton, as grown at the United States Field Station, Sacaton, Ariz.

Elderton's table,<sup>8</sup> where  $P$  is the probability that a given system of deviations from the probable may be reasonably supposed to have arisen from random sampling.

The high values of  $\chi^2$  show that the probability that the deviation of the observed frequencies from a purely random distribution is, practically speaking, infinitesimally small. In other words, extremely localized conditions inherent in the environment or in the

<sup>7</sup> PEARSON, K. Op. cit.

<sup>8</sup> ELDERTON, W. P. Op. cit.

capacities of the small groups of six seedlings are, in all of these numerous experiments, of great importance in determining seedling stand.

### SUMMARY AND CONCLUSIONS

This paper deals with the problem of the distribution of seedling stand over the agricultural field. Specifically, it indicates (1) a method for determining the theoretical number of seedlings per hill

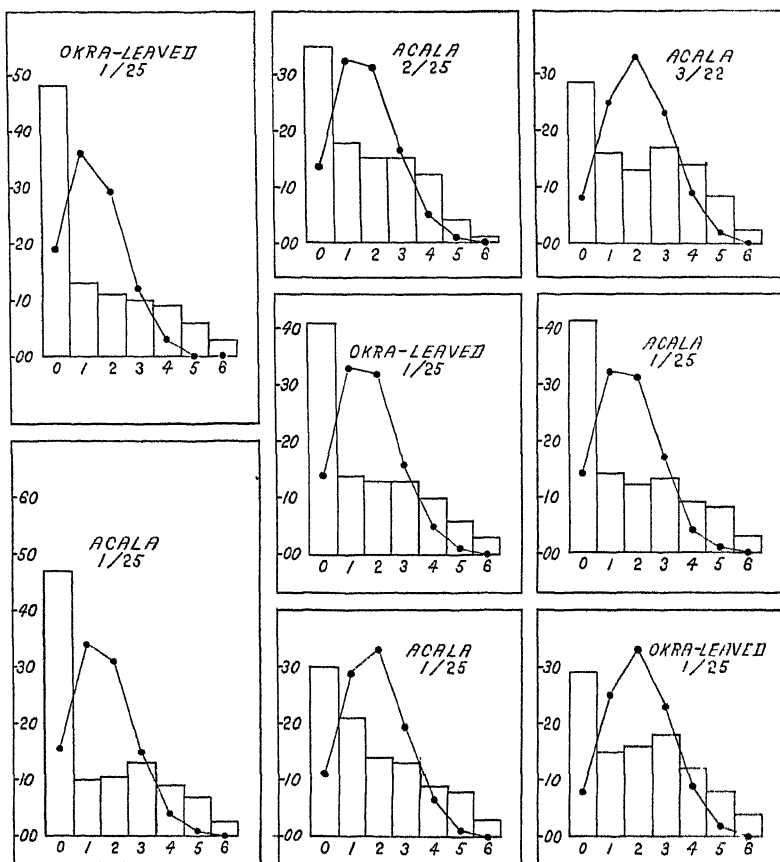


FIG. 2.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in Acala and okra-leaved Acala cotton, as grown at the United States Field Station, Sacaton, Ariz.

which should be found if the distribution were determined solely by the proportion of the seeds planted which produced plantlets and (2) a method of testing the significance of the difference between the observed and the theoretical distributions.

A suitable theoretical distribution for any field of  $N$  hills in which a constant number of seeds per hill,  $s$ , has been planted is given by the terms of  $N(p+q)^s$ , where  $p = \frac{\sum(g)}{Ns}$  is the ratio of the number of seedlings produced and standing at the period of observation to the total number of seeds planted, and  $q = 1 - p$ .



In order to determine the closeness of agreement between any experimentally determined frequency distribution of number of seedlings per hill and the theoretical distribution, some criterion of goodness of fit is required. This paper suggests the use of the  $\chi^2$  criterion for that purpose.

The results of a comparison of observed and calculated frequencies of number of seedlings per hill in an extensive series of experimental plantings of cotton on the saline soils of the Gila River Valley at

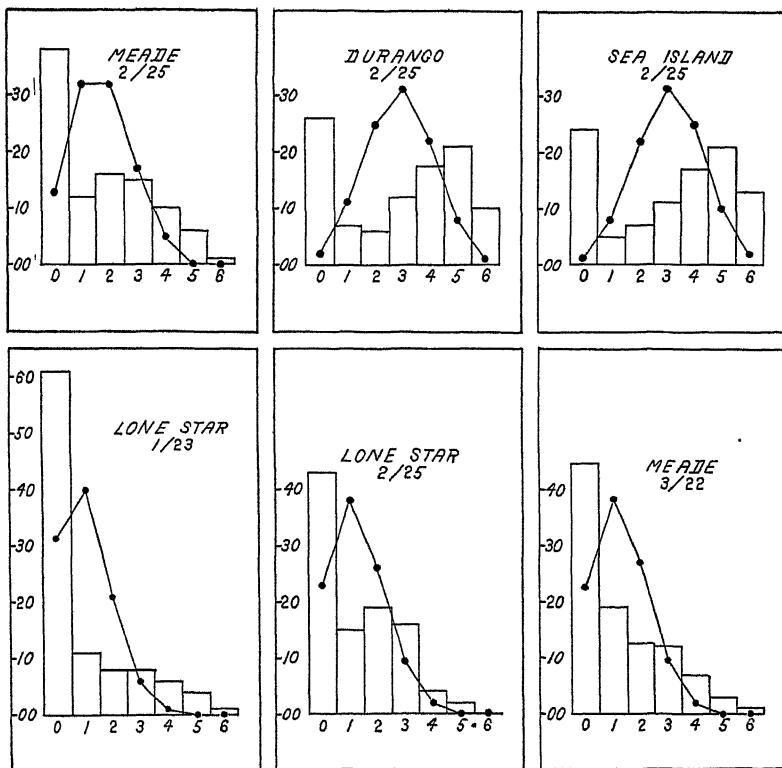


FIG. 3.—Comparison of observed (polygons) and computed (solid dots) frequencies of numbers of seedlings per hill, expressed as percentages, in sea-island cotton and in Durango, Lone Star, and Meade upland cottons, as grown at the United States Field Station, Sacaton, Ariz.

Sacaton, Ariz., show a material excess for the observed frequencies of hills without seedlings and of hills with larger number of seedlings. The application of the  $\chi^2$  test to these empirical and theoretical distributions shows that the chance of the difference between the two being due to random sampling is, practically speaking, infinitesimal.

It is clear, therefore, that the field conditions, or the samples of seeds planted in the individual hills, are highly heterogeneous with respect to factors influencing seedling stand. It is impossible, on the basis of the data presented here, to determine to what extent the observed variations in seedling stand and the observed deviations of the frequency distributions of seedling stand from the theoretical

distributions are due to soil conditions and to what extent they are due to the difficulty experienced by cotton seedlings in emerging. It is well known that, in order to secure a good stand of cotton, several seeds must be planted to a hill, since whenever the soil surface tends to form a crust the combined thrusting power of a number of seedlings is required to lift the crust and to insure the emergence of the plants. If most of the seeds planted in a particular hill chance to be nonviable, there may be too few seedlings to allow any of them to emerge. This factor is perhaps largely responsible for the excess over the expected proportion of hills containing no seedlings and of hills containing almost or quite the same number of seedlings as of seeds planted, as observed in the present investigation. But the properties of the soil which lead to the formation of a crust may differ from one part of the field to another. The peculiarities of distribution observed in this investigation may, therefore, in the last analysis, be an expression of soil heterogeneity.

That certain portions of the field may produce a better stand than others is of course quite in accord with conclusions based on general experience. As far as the writers are aware, however, quantitative tests of the significance of the deviation of an observed seedling stand from its random distribution have not heretofore been applied. Furthermore, the writers are unaware that such highly localized differences in stand as those indicated by the results here set forth have heretofore been demonstrated.

# APPLICABILITY OF PEARSON'S EQUIVALENT PROBABILITY $r$ METHOD TO THE PROBLEM OF SEEDLING MORTALITY IN SEA-ISLAND, EGYPTIAN, AND UPLAND COTTON<sup>1</sup>

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## INTRODUCTION

In the biometric attack on the problems of agriculture, one of the objectives should be the determination of the relationship between various physical factors of the environment and crop yield or quality in terms of the correlation coefficient. This has not merely the advantage of expressing the relationships in which the agriculturist is interested in quantitative, mentally comprehensible, and universally comparable terms, but also that of permitting the determination of regression equations showing the relationship between the two or more variables under consideration in the concrete terms of the units of measurement employed.

Some progress has been made in this field of research by studies dealing with the problem of the prediction of crop yields from meteorological conditions prevailing during antecedent periods of time. In investigations conducted for the Office of Alkali and Drought Resistant Plants and the Office of Western Irrigation Agriculture, of the Bureau of Plant Industry, it has been shown that the relationship between the properties of the soil and certain plant characters may be measured directly in terms of correlation (7).<sup>2</sup>

In many cases, however, it is impossible to obtain an adequate quantitative measure of the whole complex of factors which influence profoundly crop yield or quality. In such cases it is necessary to obtain some measure of the influence of these variables in terms of (a) the correlation between the characteristics of associated cultures grown under varying conditions during the same year, by employing the method of intraclass or interclass correlation (2), or in terms of (b) the correlation between the yields or other characteristics of crops grown on the same land in different years, by employing the method of interannual correlation (5).

The method of intraclass correlation (2), in the form frequently designated as the field heterogeneity coefficient (4), has been employed in agricultural investigations which have led to papers on the measurement (4) and generality (6) of field heterogeneity. In these it has been shown that irregularities in the soil influence profoundly the characteristics of the crop grown. This has been found to be true not merely for yield but for such physicochemical properties

<sup>1</sup> Received for publication Jan. 3, 1928; issued June, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 623.

of the leaf-tissue fluids as osmotic concentration and electrical conductivity (12), chloride content (13), and sulphate content (11).

Confidence in the validity of this coefficient has recently been strengthened by the demonstration that the regression of both soil properties and plant characters of (small) associated plots is, practically speaking, linear (9). While the dangers of spurious correlation were early noted (3), formulas for determining the correlation from an asymmetrical surface while still utilizing the value of the intraclass and interclass moments have recently been indicated (8).

Application of the method of interannual (5) correlation to this group of problems has shown a relatively high degree of permanence in the yield-producing capacity of small experimental plots (14), although changes, which may be referred with reasonable probability to definite physical factors, may occur in the course of time (15).

While the above-mentioned methods are the best available for dealing with plot yields, a different procedure is necessary in investigating the influence of highly localized conditions (inherent either in a small sample of seeds or in the properties of the soil, such as those affecting a single hill) on seedling stand.

In a preceding paper (10, p. 604) the effect of such conditions on seed germination has been demonstrated by means of a method which, as far as the writers are aware, has not been applied heretofore in this field of work. This involved the calculation of a theoretical distribution of the number of seedlings per hill, on the assumption that there was no correlation between the viability of the seeds of the same hill or between the environmental conditions peculiar to the individual hills and the number of seedlings produced or surviving to any given stage of development. The difference between the theoretical and the empirical frequency distributions was then expressed in terms of Pearson's  $\chi^2$  criterion (16).

The application of this method to large series of germination records for sea-island, Egyptian, and upland cotton (10) led to values of  $\chi^2$  so large as to indicate that the probability of the differences between the empirical and the theoretical distributions having arisen from random sampling of the theoretical distributions is, practically speaking, infinitesimal. This shows clearly that the individual hills are highly differentiated with respect to their capacity for the production of seedling stand.

While this method of analysis is absolutely valid and leads to fully conclusive results, it has three disadvantages. First, the values of  $\chi^2$  secured in many experiments are so large that they can not be transmuted into values of  $P$  (the probability of the given distribution having arisen from random sampling) from Elderton's tables (1, 10). Second, even if such probabilities were available in standard tables, they would be mentally incomprehensible to even highly trained individuals, the great majority of whom can not think in terms of such values as, for example,  $137/10^{35}$ . Third, such values, if laboriously computed, would be not merely incomprehensible but would express relationships in terms not directly comparable with those which have been secured in a number of other agricultural investigations.

The purpose of the present paper is to eliminate certain of the difficulties inherent in the preceding method of dealing with the problem, by applying to its analysis a procedure which brings the relationships under investigation within the scope of correlation methods (17).

#### METHODS

In an earlier paper (10) the authors determined the deviation of the actually observed frequency distribution of number of seedlings per hill from a theoretical frequency distribution. The present method also involves the deviation of observed from theoretical frequencies, but in the present case the deviations are those of the frequencies of two 2 by 2 fold tables of association.

The problem is, therefore, twofold. First, it is necessary to reduce a frequency distribution of number of seedlings per hill to a 2 by 2 fold table showing the distribution of the associations between seeds of the same hill. Second, it is necessary to determine the correlation between the fate of the seeds as potential producers of seedling stand from such a table.

In forming the fourfold table it must be noted that seeds of two categories are to be considered—those that survive to a given stage of development, *S*, and those that die before the given stage of development, *D*. Four combinations are therefore possible, *SS*, *SD*, *DS*, and *DD*. Thus the classes of the fourfold table representing the relationship of the fate of the seedlings of the same hill are as follows:

	Second seed		Total
	<i>S</i>	<i>D</i>	
First seed:			
<i>S</i> -----	<i>SS</i>	<i>SD</i>	<i>SS+SD</i>
<i>D</i> -----	<i>DS</i>	<i>DD</i>	<i>DS+DD</i>
Total-----	<i>SS+DS</i>	<i>SD+DD</i>	

Since there is no reason for regarding one seed of a hill as a first variate and another seed as a second variate of a pair, the table may be rendered symmetrical by making every possible permutation, two at a time, of the seedlings of an individual hill. Thus, if there be  $n$  seeds per hill, the number of possible permutations will be  $n(n-1)$  in number; and if  $N$  be the total number of hills, the total frequency of a table representing the experimental plot as a whole will be  $Nn(n-1)$ .

Such tables may be readily formed from a frequency distribution of the number of seedlings surviving or dying in hills in which a given number of seeds were planted, as follows: Let  $n_s$  be the number surviving and  $n_d$  the number dying per hill; then  $n_s + n_d = n$ . The contribution of hills of any class to the fourfold table will then be  $n_s(n_s-1)$  to *SS*,  $n_s n_d$  to *SD*,  $n_d n_s$  to *DS*, and  $n_d(n_d-1)$  to *DD*. These values are readily tabulated for the several classes of hills, and their total contribution to a fourfold table computed by weighting

with the class frequencies. For example, the results for 400 hills of Pima Egyptian cotton of experiment 1/25 are given in the following tabulation:

Seedlings per hill	Number of hills	$SS$ $n_s(n_s-1)$	$SD$ $n_s n_d$	$DS$ $n_d n_s$	$DD$ $n_d(n_d-1)$
0	51	0	0	0	30
1	34	0	5	5	20
2	37	2	8	8	12
3	56	6	9	9	6
4	80	12	8	8	2
5	82	20	5	5	0
6	60	30	0	0	0

Summation of these values weighted with the number of hills leads to the symmetrical 2 by 2 fold table shown below.

	$S$	$D$	Total
$S$ -----	4, 810	2, 020	6, 830
$D$ -----	2, 020	3, 150	5, 170
Total-----	6, 830	5, 170	12, 000

For a given plot the probability of survival ( $p$ ) of a seedling is given by the expression  $\frac{SS+SD}{SS+SD+DS+DD} = \frac{SS+SD}{N}$  where  $N$  is the product of the number of hills and the total permutation frequency for one hill.

It is now possible to proceed to the construction of a theoretical 2 by 2 fold table in the usual manner, by assuming the random association of one variate with the other (in this case the variate with itself), i. e., by assuming that it is associated with the other variate in the same proportion that it occurs in the total population. Hence, if the entries of the theoretical table be designated by  $S'S'$ ,  $S'D'$ ,  $D'S'$ , and  $D'D'$ , the following relations are found:

$$\begin{aligned}
 S'S' &= \frac{(SS+SD)}{N} \frac{(SS+DS)}{N} \\
 D'S' &= \frac{(DS+DD)}{N} \frac{(SS+DS)}{N} \\
 S'D' &= \frac{(SS+SD)}{N} \frac{(SD+DD)}{N} \\
 D'D' &= \frac{(DS+DD)}{N} \frac{(SD+DD)}{N}
 \end{aligned}$$

It is now possible to calculate from first principles  $\chi^2$ , which measures the deviation of the frequencies of a given table from those of the theoretical one. Thus:

$$\chi^2 = \frac{(SS-S'S')^2}{S'S'} + \frac{(DS-D'S')^2}{D'S'} + \frac{(SD-S'D')^2}{S'D'} + \frac{(DD-D'D')^2}{D'D'}$$

Now, it may be noted that interest lies in the deviation of the sample in hand from one arising from random association and not in the theoretical table as such, and inasmuch as the prime values are given in terms of the original entries, it is possible by an algebraic conversion to calculate  $\chi^2$  directly in terms of them. Thus:

$$\chi^2 = \frac{N [(SS) (DD) - (SD) (DS)]^2}{(SS + SD) (DS + DD) (SS + DS) (SD + DD)}$$

The interpretation of  $\chi^2$  is given directly by Elderton's tables (1, 18) on a probability scale, i. e., these tables give the probability that the given distribution would have arisen from a random sampling of unassociated variates. As  $\chi^2$  grows large, however,  $P$  grows less readily comprehensible, and it was for this reason that Pearson (17) translated the interpretation of the deviation to the correlation scale. In principle, the method involves merely the determination or approximate determination of a correlation coefficient which would have the same probability of arising between two unassociated variables through random sampling.

Pearson (17) has shown that if  $r_{hk}$  be the correlation between the means of the two variates of a fourfold table on an assumption of Gaussian distribution of the variates, and  ${}_o\sigma_{r_{hk}}$  its standard deviation on an assumption of absence of association, there is the following exact relationship:

$$\frac{r_{hk}^2}{{}_o\sigma_{r_{hk}}^2} = \frac{N [(SS) (DD) - (SD) (DS)]^2}{(SS + SD) (DS + DD) (SS + DS) (SD + DD)} = \chi^2$$

He has further shown that if  ${}_o\sigma_r$  designates the standard deviation of the coefficient of correlation between the two varieties (designated by " $r$ " as distinguished from the coefficient of correlation,  $r_{hk}$ , between their means) of a fourfold table, on the assumption that it is a random sample from uncorrelated material of Gaussian distribution, the following is approximately true:

$$\frac{{}_o\sigma_r}{r} = \frac{\sqrt{(SS + SD) (DS + DD) (SS + DS) (SD + DD)}}{\sqrt{N} [(SS) (DD) - (SD) (DS)]}$$

or approximately

$$\chi^2 = \frac{N [(SS) (DD) - (SD) (DS)]^2}{(SS + SD) (DS + DD) (SS + DS) (SD + DD)} = \frac{r^2}{{}_o\sigma_r^2}$$

Since interest centers in the association of the variates and not their means, it is necessary to deal with the latter relationship.

In determining the value of  $r$  from the relationship between  $\chi^2$  and  $r^2/{}_o\sigma_r^2$ , it is possible to take  ${}_o\sigma_r = \frac{1}{\sqrt{N}} \times \chi_{\alpha_1} \times \chi_{\alpha_2}$ , as given by a fourfold Gaussian table.  $\chi_{\alpha_1}$  and  $\chi_{\alpha_2}$  are the corrections of  ${}_o\sigma_r$  for the failure of the table to have the material in equal-ranged cells. Here it is not necessary to deal with their technical significance any further than to point out that they are functions of  $\frac{1}{2}(1 + \alpha_1)$ ,  $\frac{1}{2}(1 - \alpha_1)$ ,  $\frac{1}{2}(1 + \alpha_2)$ , and  $\frac{1}{2}(1 - \alpha_2)$  where  $\frac{1}{2}(1 + \alpha_1)$  and  $\frac{1}{2}(1 - \alpha_1)$

represent the two areas into which the frequencies of the variable designated by subscript 1 are divided when the total frequency is reduced to unity. In the present problem the variables are identical. Thus:  $\alpha_1 = \alpha_2$ , and the division is between  $S$  and  $D$ .

$$\begin{aligned} \text{Thus } \frac{1}{2}(1 + \alpha) &= p \text{ if } p \geq 0.5 \\ &= 1 - p = q \text{ if } p \leq 0.5 \\ \text{and } \frac{1}{2}(1 - \alpha) &= 1 - p = q \text{ if } p \geq 0.5 \\ &= p \text{ if } p \leq 0.5 \end{aligned}$$

For values of  $\frac{1}{2}(1 + \alpha)$  given by a fourfold table, one may read off the corresponding  $\chi^2$ 's from Pearson's Table V (17) reprinted from *Biometrika*. For values of  $\frac{1}{2}(1 + \alpha)$  beyond two decimal places, it is necessary to interpolate between two tabular values of  $\chi^2$  to determine the desired value.

With the value  ${}_0\sigma_r$  thus determined and the value  $\chi^2$  calculated, it is possible to determine a value " $r$ ," which will satisfy the approximation

$$\chi^2 = \frac{r^2}{{}_0\sigma_r^2}$$

For this purpose Pearson has published two tables, one involving  $\chi^2$  and the other  $\log \chi^2$ , of which the former, Table III (17), which gives values of  $\chi^2$  corresponding to values of  $r$  and  ${}_0\sigma_r$ , will be used here. In practice, this determination of  $r$  involves a double interpolation, one for values of  $\chi^2$  to correspond to values of  ${}_0\sigma_r$  beyond two decimal places, and thereafter another interpolation between two tabulated values of  $r$  to obtain the one corresponding to a given  $\chi^2$ . This procedure will be clear from a numerical example to follow.

The correlation coefficient thus obtained is an approximation of the tetrachoric  $r$  of the fourfold table, and subject to the same interpretation, with allowances. Allowance must be made not only for the approximation involved in the theory but for approximations involved in interpolation. There can be no doubt, however, that in spite of the approximations, this  $r$  presents to the mind a much more accurate impression of the degree of association than does the function  $P$ , representing the probability of there being no association. For purposes of distinction, and in view of its derivation and purpose, this correlation coefficient may be termed the "equivalent probability  $r$ ."

As an example consider the "equivalent probability  $r$ " for the 400 hills of the plot of Pima Egyptian cotton in experiment 1/25, for which the tabulation on page 618 gives the fourfold distribution.

To calculate directly:

$$\begin{aligned} \chi^2 &= \frac{N[(SS)(DD) - (SD)(DS)]^2}{(SS + SD)(DS + DD)(SS + DS)(SD + DD)} \\ &= \frac{12000(11071100)^2}{(6830)(5170)(6830)(5170)} = 1179.6127 \end{aligned}$$

$$\frac{1}{2}(1 + \alpha_1) = \frac{1}{2}(1 + \alpha_2) = \frac{1}{2}(1 + \alpha) = p = \frac{SS + SD}{N} = \frac{6830}{12000} = 0.569$$



Interpolating for  $\chi_\alpha$  one has:

$$\begin{array}{cc} \frac{1}{2}(1+\alpha) & \chi_\alpha \\ 0.56 & 1.2585 \\ 0.569 & 1.2602 \\ 0.57 & 1.2604 \end{array} \quad \chi_\alpha = 1.2585 + 0.9(0.0019) = 1.2602$$

$$\begin{aligned} {}_o\sigma_r &= \frac{1}{\sqrt{N}} \chi_{\alpha_1} \chi_{\alpha_2} = \frac{1}{\sqrt{N}} \chi_\alpha^2 = \frac{1}{\sqrt{12000}} \times (1.2602)^2 \\ &= 0.009129 \times 1.588104 = 0.0145 \end{aligned}$$

Interpolating values of  $\chi^2$  for  ${}_o\sigma_r = 0.0145$  for values of  $r = 0.4$  and  $r = 0.5$ , the values as indicated in the table opposite that entry are obtained.

${}_o\sigma_r$	$\chi^2(r=0.4)$	$\chi^2(r=0.5)$
0.01	1758.21	2892.33
0.0145	1168.53	1920.19
0.02	447.81	731.95

Interpolation for the  $r$  corresponding to  $\chi^2 = 1179.61$ , gives:

$$\begin{array}{cc} \chi^2 & r_p \\ 1168.53 & 0.4 \\ 1179.61 & \\ 1920.16 & 0.5 \end{array} \quad r_p = 0.4 + \frac{(0.1) 11.08}{751.63} = 0.4015$$

#### MATERIAL

The materials on which this study is based have been described in detail in another paper (10). The data comprise records of cotton stands from five different experiments with Pima Egyptian, sea-island, and upland cotton.

#### RESULTS

The results of the present rather onerous investigation may be condensed into a single accompanying table. (Table I.) In this the original experiment numbers are retained. Reference to the frequency distributions of number of seeds per hill, as published in an earlier paper (10), is made by number in the second column. The number of hills appears in the third column. The permutations of seeds which survived and of those which died are indicated by the weighted frequencies in the fourth to seventh columns. The values of  $\chi^2$  appear in the eighth column, and the values of the equivalent probability  $r$ , as deduced from these values of  $\chi^2$ , are given in the final column of the table.

For present purposes it has seemed unnecessary to consider the probable errors of these correlations. Without laying too much emphasis on the significance of the individual values of  $r_p$ , it is clear that these constants have material values. They range from  $r = 0.31$  to  $r = 0.75$ . Thus there is at least a medium correlation between the fate of seedlings of the same hill.

TABLE 1.—*Permutation frequencies of number of seeds which survived (S), and number of seeds which died (D), in hills of sea-island, Egyptian, and upland cotton, together with values of  $\chi^2$  and equivalent probability correlation coefficients measuring the relationship between the fate of seedlings of the same hill*

Experiment and variety	Table No. (10)	Number of hills (N)	Survived and survived (SS)	Survived and died (SD)	Died and survived (DS)	Died and died (DD)	$\chi^2$	r
Experiment 3/22								
Pima Egyptian	3	1,440	8,188	6,032	6,032	22,948	5,839.7	0.6599
Meade upland	3	1,440	4,032	5,413	5,413	28,342	3,068.9	.5111
Acala upland	3	1,440	7,894	7,071	7,071	21,164	3,316.2	.5267
Experiment 1/23								
Pima Egyptian	4	1,440	4,614	3,791	3,791	31,004	8,363.7	.7463
Lone Star upland	4	1,440	3,780	3,770	3,770	31,870	6,758.8	.7062
Experiment 1/25:								
Pima Egyptian	5	720	5,916	3,354	3,354	8,976	2,896.1	.5118
Smooth-seeded Pima Egyptian	5	720	9,038	2,987	2,987	6,588	4,175.0	.5976
Acala upland	5	720	3,140	2,600	2,600	13,260	3,170.2	.5590
Okra-leaved Acala upland	5	720	2,734	2,506	2,506	13,854	2,934.3	.5516
Pima Egyptian	6	400	4,810	2,020	2,020	3,150	1,179.6	.4015
Smooth-seeded Pima Egyptian	6	400	5,378	1,842	1,842	2,938	1,551.0	.4556
Acala upland	6	400	1,902	1,838	1,838	6,422	981.8	.3908
Okra-leaved Acala upland	6	400	2,148	1,962	1,962	5,928	921.5	.3683
Pima Egyptian	5, 6	1,120	10,726	5,374	5,374	12,120	4,333.4	.5908
Smooth-seeded Pima Egyptian	5, 6	1,120	14,416	4,829	4,829	9,526	5,722.2	.6547
Acala upland	5, 6	1,120	5,042	4,438	4,438	19,082	4,065.8	.5740
Okra-leaved Acala upland	5, 6	1,120	4,882	4,468	4,468	19,782	3,836.1	.5595
Experiment 2/25:								
Pima Egyptian with Meade upland	7-10	240	2,054	1,196	1,196	2,754	780.4	.4370
Pima Egyptian with Lone Star upland	7-10	240	2,250	1,165	1,165	2,620	887.4	.4590
Pima Egyptian with Acala upland	7-10	240	2,434	1,306	1,306	2,154	538.0	.3635
Pima Egyptian with Durango upland	7-10	240	2,252	1,273	1,273	2,402	615.9	.3902
All Pima Egyptian	7-10	960	8,990	4,940	4,940	9,930	2,824.3	.4940
Sea island with Meade upland	7-10	240	2,520	975	975	2,730	1,509.4	.5788
Sea island with Lone Star upland	7-10	240	2,726	1,084	1,084	2,300	1,127.5	.5124
Sea island with Acala upland	7-10	240	2,784	1,126	1,126	2,164	984.5	.4870
Sea island with Durango upland	7-10	240	2,656	919	919	2,706	1,724.6	.6113
All sea island	7-10	960	10,686	4,104	4,104	9,906	5,314.8	.6368
Meade upland	7-10	240	986	1,069	1,069	4,070	532.8	.4385
Lone Star upland	7-10	240	552	1,008	1,008	4,632	220.8	.3100
Acala upland	7-10	240	922	1,113	1,113	4,052	406.4	.3898
Durango upland	7-10	240	2,452	1,043	1,043	2,662	1,270.4	.5372

## SUMMARY AND CONCLUSIONS

Earlier studies have dealt with the problem of the significance of the deviation of number of seedlings produced per hill in sea-island, Egyptian, and upland cotton, from the theoretical distributions which would result if the distribution of the stand were a purely random one.

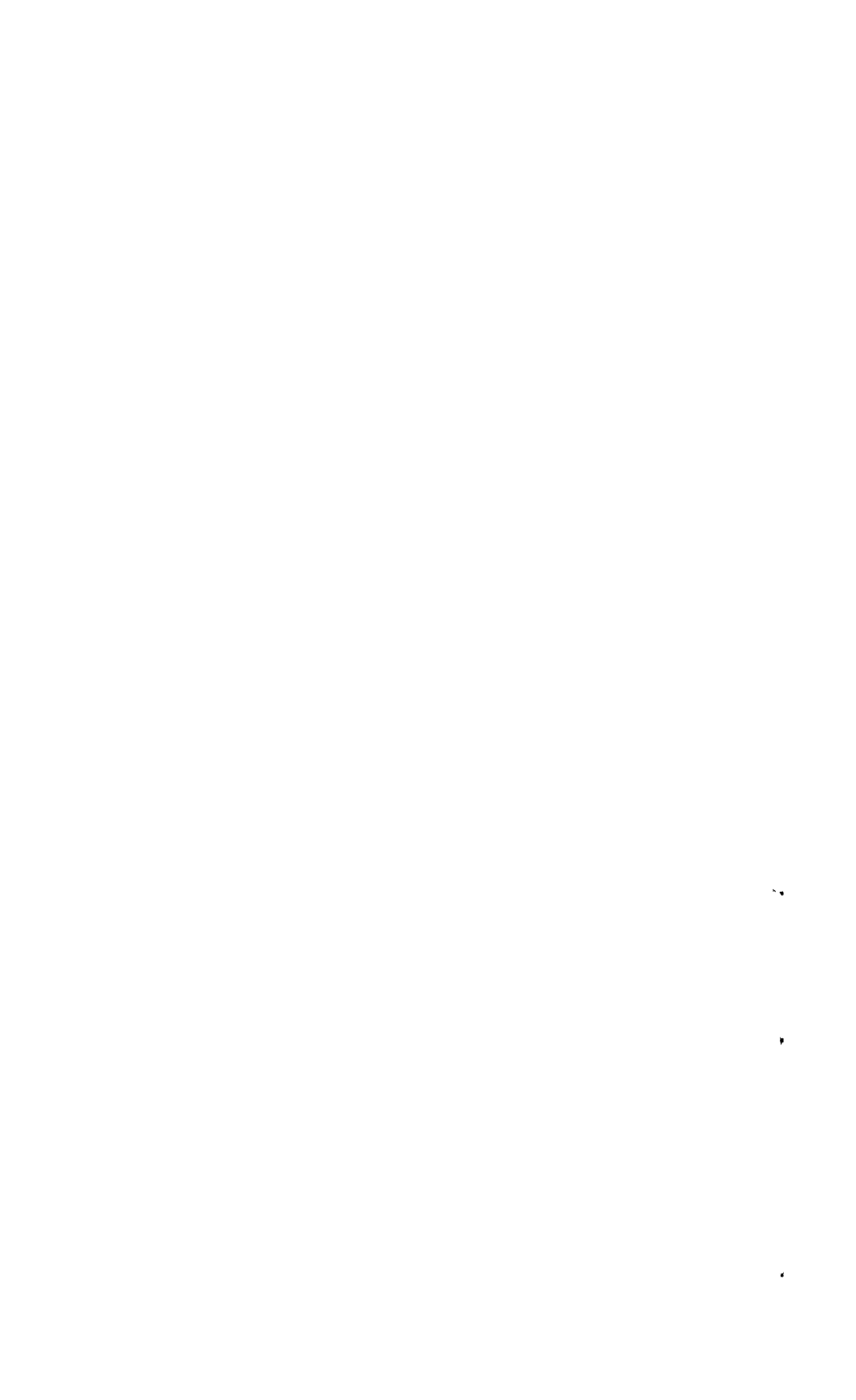
The present paper indicates methods by which the deviations of the stand from a purely random distribution may be translated into terms of correlation between the fate of the seedlings of the same hill.

The stands obtained in a wide series of experiments show that there is a medium correlation between the fate of the seeds of the same hill. Thus, either extremely localized conditions or random differences in the capacities of the seeds of the various hills for establishing themselves as seedlings play a large rôle in determining variation in seedling stand.

The advantages of this method of attack are twofold. First, it expresses relationships in terms of correlations which are more readily comprehensible than those of large values of  $\chi^2$  and almost infinitesimally small values of  $P$ . Second, it permits the extension to this field of work of a combination of methods which have heretofore been employed in a wide range of field heterogeneity investigations.

## LITERATURE CITED

- (1) ELDERTON, W. P.  
1902. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. *Biometrika* 1: 155-163.
- (2) HARRIS, J. A.  
1913. ON THE CALCULATION OF INTRA-CLASS AND INTER-CLASS COEFFICIENTS OF CORRELATION FROM CLASS MOMENTS WHEN THE NUMBER OF POSSIBLE COMBINATIONS IS LARGE. *Biometrika* 9: 446-472.
- (3) ———  
1914. ON SPURIOUS VALUES OF INTRA-CLASS CORRELATION COEFFICIENTS ARISING FROM DISORDERLY DIFFERENTIATION WITHIN THE CLASSES. *Biometrika* 10: 412-416.
- (4) ———  
1915. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. *Amer. Nat.* 49: 430-454, illus.
- (5) ———  
1915. THE VALUE OF INTER-ANNUAL CORRELATIONS. *Amer. Nat.* 49: 407-712.
- (6) ———  
1920. PRACTICAL UNIVERSALITY OF FIELD HETEROGENEITY AS A FACTOR INFLUENCING PLOT YIELDS. *Jour. Agr. Research* 19: 279-314.
- (7) ———  
1926. THE RELATIONSHIP BETWEEN THE CONCENTRATION OF THE SOIL SOLUTION AND THE PHYSICOCHEMICAL PROPERTIES OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. *Jour. Agr. Research* 32: 605-647, illus.
- (8) ———  
1927. ON THE ELIMINATION OF SYMMETRY AS A SOURCE OF SPURIOUS VALUES OF THE FIELD HETEROGENEITY COEFFICIENT. *Minn. Univ. Studies Biol. Sci.* 6: 343-350.
- (9) ——— CONNORS, I. L., ELDERS, A. T., and KIRK, L. E.  
1927. ON THE REGRESSION OF SOIL PROPERTIES AND CROP CHARACTERS IN ASSOCIATED PLOTS OF AN EXPERIMENTAL FIELD. *Minn. Univ. Studies Biol. Sci.* 6: 351-371, illus.
- (10) ——— HARRISON, G. J., and WADLEY, F. M.  
1928. ILLUSTRATIONS OF THE APPLICATION OF A CRITERION OF THE DEVIATION OF AN OBSERVED FROM A RANDOM DISTRIBUTION TO THE PROBLEM OF SEEDLING STAND IN SEA ISLAND, EGYPTIAN, AND UPLAND COTTON. *Jour. Agr. Research* 36: 603-614, illus.
- (11) ——— HOFFMAN, C. T., and HOFFMAN, W. F.  
1925. SULPHATE CONTENT OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. *Jour. Agr. Research* 31: 653-661.
- (12) ——— LAWRENCE, Z. W., HOFFMAN, W. F., LAWRENCE, J. V., and VALENTINE, A. T.  
1924. THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F<sub>1</sub> HYBRID. *Jour. Agr. Research* 27: 267-328, illus.
- (13) ——— LAWRENCE, J. V., and LAWRENCE, Z. W.  
1924. THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. *Jour. Agr. Research* 28: 695-704.
- (14) ——— and SCOFIELD, C. S.  
1920. PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD. *Jour. Agr. Research* 20: 335-356.
- (15) ——— and SCOFIELD, C. S.  
1928. FURTHER STUDIES ON THE PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD. *Jour. Agr. Research* 36: 15-40, illus.
- (16) PEARSON, K.  
1900. ON THE CRITERION THAT A GIVEN SYSTEM OF DEVIATIONS FROM THE PROBABLE IN THE CASE OF A CORRELATED SYSTEM OF VARIABLES IS SUCH THAT IT CAN BE REASONABLY SUPPOSED TO HAVE ARISEN FROM RANDOM SAMPLING. *Phil. Mag.* 50: 157-175.
- (17) ———  
1912. ON A NOVEL METHOD OF REGARDING THE ASSOCIATION OF TWO VARIATES CLASSED SOLELY IN ALTERNATE CATEGORIES. *Drapers' Co. Research Mem. Biometric Ser.* 7, 29 p., illus.
- (18) ———  
1924. TABLES FOR STATISTICIANS AND BIOMETRICIANS. Ed. 2, part 1. London.



# NUTRITIONAL STUDIES ON THE SEED-CORN MAGGOT, *HYLEMYIA CILICRURA* RONDANI<sup>1</sup>

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## INTRODUCTION

The seed-corn maggot, *Hylemyia cilicrura* Rondani, is a pest of various crops, such as corn, beans, and potatoes, during the period of seed germination. The following investigation on the nutrition of the larva of this species sheds some particularly useful light on the question of the exact rôle of bacteria in the nutrition of the fly larva and clears up a question of much economic importance in regard to the biology of the species. From the economic point of view it was desired to learn whether or not the action of bacteria must precede the attack of the larva upon potato seed pieces, sprouting corn, and other plants susceptible to attack by the insect. From the theoretical standpoint it is important to know more about the food substances which are essential to larval development and which are made available by microorganisms.

## HISTORICAL

Loeb and Northrop<sup>3</sup> have clearly shown the importance of yeasts in the nutrition of *Drosophila* larvae. They thought that yeasts were the indispensable food of these flies, and they proved that the value of the yeasts lay in their synthetic powers, for the larvae were able to live upon either living yeasts or yeasts which had been subjected to heat of 120° C. for one hour. Their attempts to extract the necessary substance from the yeast met with failure, and they were at a loss to explain the exact nature of this substance. Northrop<sup>4</sup> investigated the matter further and found that the specific substance essential to growth was also present in the kidneys, liver, and pancreas of the dog, in the liver of the mouse, and in the bodies of the flies themselves.

Leach<sup>5</sup> made similar studies on *Hylemyia cilicrura* (under the name *Phorbia fusciceps* Zett.). He found that bacteria were important in the normal development of these larvae and, in fact, went so far as to say that bacteria are essential for the development of the maggots. He found further that bacteria of the substratum, including some pathogenic species, when taken up by the larva of *Hylemyia cilicrura*, may live through the pupal stage and be disseminated by the adult fly. Since this condition makes possible the continuous associa-

<sup>1</sup> Received for publication Feb. 3, 1928; issued June, 1928.

<sup>2</sup> This investigation was conducted by the writer during the summer of 1927, while he was stationed at Chadbourn, N. C., as a temporary employee of the Bureau of Entomology. The writer wishes to express his thanks to W. H. White and to W. J. Reid, jr., for valuable aid in this work.

<sup>3</sup> LOEB, J., and NORTHROP, J. H. NUTRITION AND EVOLUTION. SECOND NOTE. Jour. Biol. Chem. 27: 309-312. 1916.

<sup>4</sup> NORTHROP, J. H. THE RÔLE OF YEAST IN THE NUTRITION OF AN INSECT (*DROSOPHILA*). Jour. Biol. Chem. 30: 181-187. 1917.

<sup>5</sup> LEACH, J. G. THE RELATION OF THE SEED-CORN MAGGOT (*PHORBIA FUSCICEPS* ZETT.) TO THE SPREAD AND DEVELOPMENT OF POTATO BLACKLEG IN MINNESOTA. Phytopathology 16: 149-176, illus. 1926.

tion of the maggot with the bacterial flora of the substratum, it appeared interesting and important to investigate further the food requirements of the larva with special reference to the rôle played by the bacteria present.

## PRESENT INVESTIGATIONS

### FOOD REQUIREMENTS OF THE LARVAE

Leach's conclusions were based upon experiments with sterile larvae placed upon sterile potato plugs and upon beef-extract agar. He observed that sterile maggots were unable to grow on sterile potato plugs, but that they grew and pupated on potato plugs which had been inoculated with bacteria found naturally in their environment. Repetition of Leach's experiments by the author yielded like results. No larvae grew to maturity on either of the two kinds of media when sterility was obtained. Normal growth and pupation occurred in a certain percentage of cases in which the potato plug was contaminated. The larvae grew on nonsterile beef-extract agar, but always died before pupation.

It was desired to know whether the inability of the larva to live upon sterile potato plugs was due to the destruction of enzymes by the high temperatures used in sterilization. To determine this a bacteria-free filtrate, obtained by passing the fluid from macerated tubers through a Berkefeld filter, was added to the sterile potato plugs. Repeated attempts to grow larvae on this medium were unsuccessful.

### BACTERIA AS FOOD FOR THE LARVAE

Upon the supposition that the larvae might live upon bacteria which had been killed by heat, a heavy suspension of killed bacteria was added to sterile potato plugs. The organisms used were obtained by inoculating agar slants from cultures in which larvae grew normally, and, after an incubation period of 24 hours, making a suspension of the resulting growth in isotonic salt solution. Attempts to grow larvae on this medium were unsuccessful. There still remained the possibility that the heating of the bacteria destroyed some essential substance. Suspensions of living bacteria were filtered, using a Berkefeld filter, and the filtrate was added to a suspension of killed bacteria. Attempts to grow larvae on sterile potato plugs to which this mixture was added were also unsuccessful.

### RELATION OF BACTERIA TO THE NUTRITION OF THE LARVA

After these experiments, it began to appear that the part played by the bacteria in the life of the larvae must be a secondary one, and that it probably consisted of the effects of the bacteria upon the substratum rather than of any peculiar qualities of the bacteria themselves. Hence, in the next experiments, potato plugs were inoculated with bacteria and incubated for several days until they were covered with an abundant, slimy growth. They were then sterilized by heat. When newly hatched, sterile larvae were placed upon this medium they grew rapidly, pupated normally, and produced adults which were normal in appearance. These larvae, pupae, and adults were all tested for bacterial sterility and were found to be sterile. These experiments proved conclusively that the

fly could live from egg to adult without the presence of living bacteria; and they indicated that the rôle of the bacteria was merely that of an agent for converting the potato tuber into available food for the larvae. These experiments were then modified by using fermented and partially decomposed beans and peas, which were later sterilized by heat. It was found that this latter medium was more satisfactory for routine use than that made from potato. A stock of it was thereafter kept on hand and used for rearing newly hatched larvae as soon as they had been tested for sterility. In this way it was possible to have on hand for other experiments sterile larvae of various known ages and of the corresponding sizes.<sup>6</sup>

#### ATTEMPTS TO GROW LARVAE IN THE ABSENCE OF BACTERIA

Having found that the larvae did not require living bacteria for their development, the writer believed that a method might be found for growing them in complete absence of any microorganisms, either dead or living. Upon the supposition that the action of the bacteria in making the substance of potatoes and beans available for use by the larvae consisted of some kind of digestion, various attempts were made to produce these changes without the presence of bacteria. Artificial digestion by saliva and by artificial pancreatic juice were first undertaken. Fresh saliva was filtered through a Berkefeld filter to obtain a filtrate free from bacteria, and this was added to sterile potato. The artificial pancreatic juice was prepared by using a powdered extract of pancreas. The powdered product was mixed with water and filtered through a Berkefeld filter. The bacteria-free filtrate was added to sterile potato. Attempts to grow larvae on such media were not successful. The addition of sterile dextrose water to the sterile potato plugs also failed to provide a suitable medium for growth.

In the next attempts to grow the larvae advantage was taken of the natural processes of digestion which occur when seeds germinate. As these digestive changes may go on in the absence of bacterial action, efforts were made to grow various seedlings to be used as a medium for larval growth under conditions of bacterial sterility. No very satisfactory method for obtaining a high percentage of bacterially sterile seedlings from a given number of seeds was found. Simple treatment of the seeds with bichloride of mercury solution (1:1,000) for 15 minutes, with subsequent rinsing with sterile water, gave a small percentage of sterile seedlings. After treatment the seeds were placed on sterile agar and allowed to germinate, and any showing growth of bacteria or molds within a week were discarded. When a seedling was found to be bacterially sterile, a sterile larva was placed upon it and observed from day to day. It was found that these larvae attacked the roots of sterile seedlings of corn, beans, peas, and radishes, and grew rapidly while living upon this medium. They pupated in the normal length of time, and the pupae produced adults which were normal in appearance. Sterility tests were made upon the larvae, pupae, and adults, and all of these stages failed to

<sup>6</sup> It should be said here that Bogdanov's<sup>7</sup> method of obtaining sterile larvae by treating the eggs with bichloride of mercury solution (1:1,000) for 15 minutes was found to be a very satisfactory routine method and was followed throughout these experiments.

<sup>7</sup> BOGDANOV, E. A. ÜBER DIE ABHÄNGIGKEIT DES WACHSTUMS DER FLIEGENLARVEN VON BAKTERIEN UND FERMENTEN UND ÜBER DIE VARIABILITÄT UND VERÄNDERUNG BEI DEN FLEISCHFLIEGEN. Arch. Anat. u. Physiol., Physiol. Abt., 1908 (sup): 173-200. 1908.

produce bacterial growth when smeared on agar slants or crushed in beef-dextrose broth. By transferring the larvae from the sterile, decomposed bean medium it was possible to place a sterile larva at any stage of its life upon the seedling. When observed under the microscope the larva was seen to use its mouth hooks in tearing the plant tissue into shreds. No part of the bean seedling seemed to be exempt from attacks from the larva, but the cotyledons and leaves were usually not attacked until the larva had almost reached maturity. (Fig. 1, C and D.)

The procedure followed in testing for sterility was to allow the larva to pupate in the tube containing the seedling, then to transfer the puparium to another agar-slant tube into which had been placed a folded piece of sterile filter paper. The original tube was then incubated for 24 hours and examined. When the adult emerged, it was allowed to crawl over the surface of the agar and to deposit feces thereon. It was then removed and crushed in a tube of sterile dextrose beef broth. The tube containing the empty puparium and the broth tube were then incubated for 24 hours and examined for sterility. Control tests were run upon the corresponding stages of flies which were grown under nonsterile conditions. Such control tests always showed abundant bacterial growth while the other cultures remained sterile. (Fig. 1, A and B.)

It should be noted that the possibility that living symbionts may have been present in the egg and were passed to and continued to live in the later stages has not been disproved by these experiments, since these entities do not grow on ordinary bacteriological media.

#### ESSENTIAL FOOD SUBSTANCES

The results of these studies indicate that substances essential to life for *Hylemyia cilicrura* larvae may be obtained from potato plugs and from beans, peas, and other seeds following the growth of bacteria upon these media; and that suitable substances are also present in growing bean and other seedlings which have been free from bacterial action.

#### SUMMARY AND CONCLUSIONS

Leach's observations that sterile larvae do not grow to maturity on sterile beef-extract agar or potato plugs, whereas larvae grow normally on contaminated potato plugs, are confirmed by these studies.

Experiments using bacteria-free filtrate from unheated potatoes indicate that the failure of larvae to grow on heated potato is not due to the destruction of thermolabile substances such as enzymes.

Attempts to grow larvae on sterile potato to which had been added a suspension of heated bacteria were unsuccessful.

Potato plugs, beans, and peas which had partially decomposed and then were sterilized by heat proved to be good media for growth. Flies were reared from egg to adult on such media and found to be bacterially sterile.

Attempts to grow larvae on potato which had been submitted to artificial digestion by saliva and pancreatic juice were unsuccessful.

Growing bean and pea seedlings free from bacteria were found to provide a suitable medium for growth, flies being reared from egg to adult on such seedlings without the presence of bacteria, either dead or living.



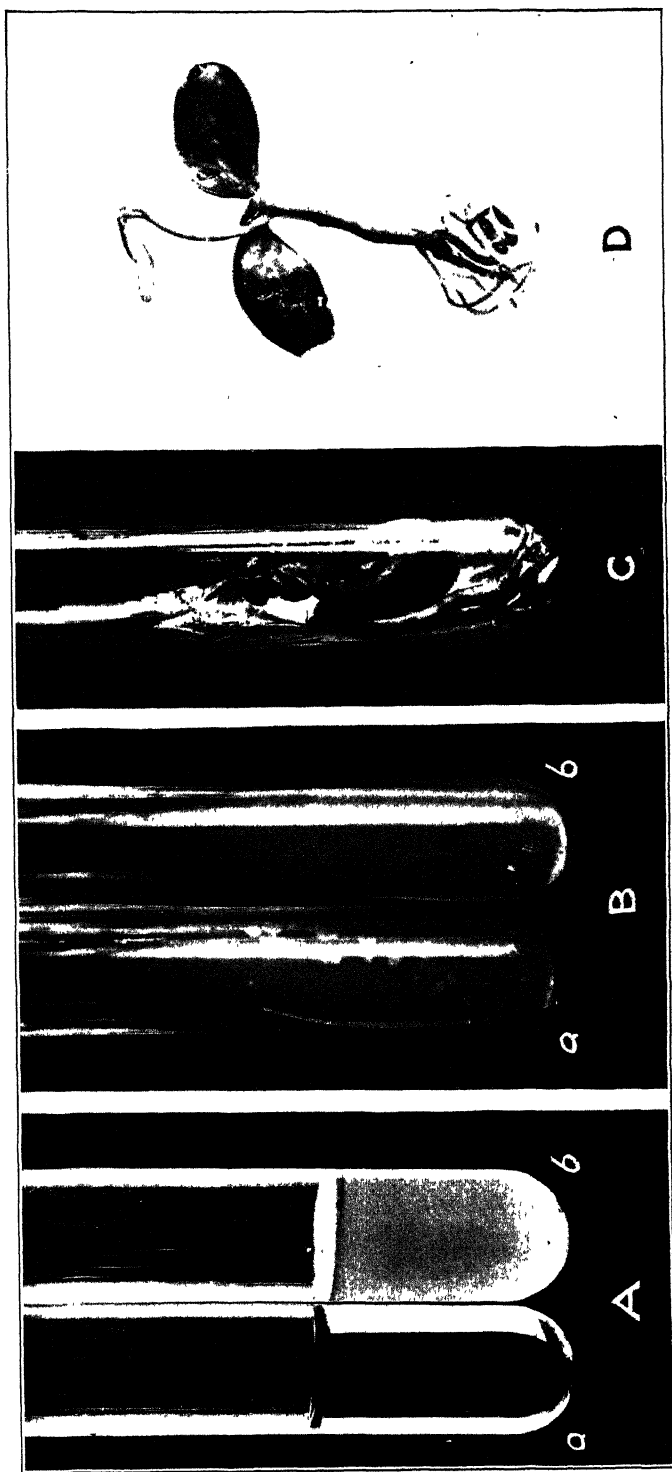


FIG. 1.—A, Broth culture containing a sterile larva (a); broth culture (control) containing an ordinary larva (b). B, Agar slants upon which puparia were placed. The adults emerged and crawled over the surface of the agar. The control tube (a) contained a puparium from an ordinary larva; the other tube (b) contained a puparium from a sterile larva. C, Sterile larva on sterile bean seedling. The larva attained full growth in nine days and pupated. Imprints of the mouth parts on the agar remained sterile. D, Bean seedling from the tube in C, showing injury to the cotyledons done by the sterile larva.

From the results obtained in these experiments it seems necessary to conclude that the presence of bacteria, per se, is not essential to the development and pupation of the larvae of *Hylemyia ciliocrura*. It seems permissible to conclude also that in the nutrition of the larvae of this species, the bacteria by their action on the medium sometimes play the rôle of preparing a suitable substratum for growth of the larva.

It seems permissible to conclude also that the substances essential to the growth of these larvae are present in bacteria-free, growing seedlings of beans and some other seeds.

# THE OCCURRENCE AND BEHAVIOR OF EMBRYOLESS WHEAT SEEDS<sup>1</sup>

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## INTRODUCTION

The occurrence of wheat caryopses entirely devoid of embryos but perfectly normal as to endosperm development has not previously been reported. Harlan and Pope (11),<sup>3</sup> working with barley, are the only experimenters who record data concerning a similar phenomenon. They attribute this abnormality to single fertilization. Evidently, according to these workers, only the endosperm fertilization takes place in the development of the embryoless seed. Wheat seeds exhibiting this characteristic have been observed many times by the writer during the course of a study upon the respiration of seeds injured by threshing. It has also been found that such embryo-lacking seeds respire and give evidence of enzyme activity.

## OCCURRENCE OF SEEDS WITHOUT EMBRYOS

The rarity of this abnormal development in barley, as reported by Harlan and Pope (11), was not found by the writer to be equally apparent in wheat. These workers found only five seeds completely devoid of embryo tissue among many thousands of barley seeds examined, while the writer observed the occurrence of such seeds in each lot of wheat received at the Colorado seed laboratory during the past year. To obtain enough material with which to conduct experiments on respiration and enzyme activity it was necessary to examine minutely large numbers of wheat seeds. In connection with this careful scrutiny of some 150,000 wheat caryopses it was estimated that approximately 0.1 per cent of the seeds of 20 different lots of wheat had developed no embryo. The lots of wheat examined were representative of various varieties of both spring and winter wheats, specifically, Kanred, Turkey Red, Kota, Defiance, and Marquis. It appears from the above that the occurrence of embryo-lacking seeds in wheat is neither uncommon nor limited to one variety.

Embryo-free seeds closely resemble normal seeds, but upon careful examination a deep depression may be noted at the point where embryo development normally occurs. Figures 1, 2, and 3 illustrate the appearance of the embryo-deficient seeds as compared with normal seeds. In Figure 3 it can be readily seen that no embryo development has taken place.

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<sup>1</sup> Received for publication Jan. 30, 1928; issued June, 1928.

<sup>2</sup> The writer wishes to express her grateful appreciation to Anna M. Lute, State seed analyst, for her valuable suggestions and criticisms during the progress of the entire work, and to Dr. L. W. Durrell, professor of botany, Colorado Agricultural College, for his helpful assistance with the manuscript.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 637.

## RESPIRATION AND ENZYME ACTIVITY OF EMBRYOLESS SEED

While it is obvious that an embryo-free seed will not grow when subjected to conditions suitable for germination, the absence of the embryo in these abnormal seeds suggests the problem of determining whether or not such seeds exhibit vital processes which accompany germination of the normal seed, such as enzyme activity and respiration. Accordingly a study was made of the catalase activity and the liberation of  $\text{CO}_2$  through the respiratory process as a means of determining the activity of those seeds which develop no embryo.

Various investigators have attributed the secretion of enzymes in the Gramineae to three different sources: (1) To the scutellum, (2) to the aleurone layer, and (3) to the amylaceous cells of the endosperm. The secreting power of the epithelial layer of the scutellum in greater or less degree has been noted by practically all investigators, some going so far as to ascribe all secretion to that organ alone.

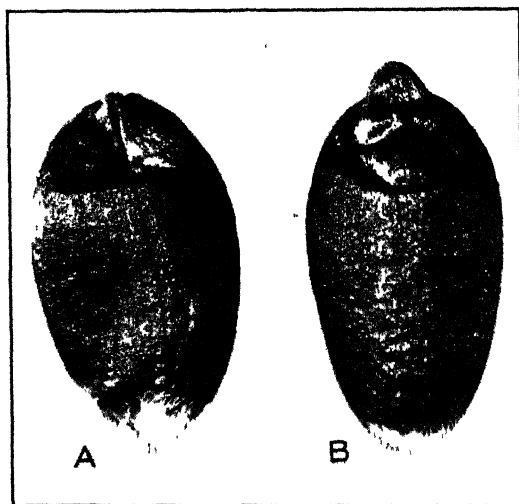


FIG. 1.—A, a wheat seed which has developed without an embryo as compared with B, a normal seed; note the deep indentation at the embryo end of the abnormal seed

Mann and Harlan (15) and Brown and Morris (6) from their physiological studies made on barley, and Torrey (21), working on germinating maize, conclude that the entire source of diastase and catalase during germination is localized in the epithelial layer of the scutellum. Working with Stoner wheat, Crocker and Harrington (10) found that the catalase activity of the embryo is twenty-eight to twenty-nine times greater than that of the endosperm. Bailey and Gurjar (4) consider the wheat embryo the seat

of respiration. They also state that it is very much richer in enzymes than is the endosperm, and conclude that respiration is decidedly greater in the embryo if not wholly confined to it.

From his experiments with whole seeds and excised embryos, Burlakov (8) concludes that wheat embryos respire much more vigorously, weight for weight, than do entire seeds, the respiratory ratio being 1 to 16. This conclusion is also supported by observations of Karchevski (13) who states that in the wheat embryo the rate of respiration is twelve times greater than in the entire seed. According to Kolkwitz (14) the half of a wheat seed containing the embryo respire three times as much  $\text{CO}_2$  as the distal half when equal weights of material are compared.

Fewer investigators favor the theories of endosperm secretion which assign vital activity to either the aleurone layer or the starchy endosperm. Bruschi (7), experimenting with several species of the Gramineae, maintains that the amylaceous endosperm can digest

itself in varying degrees in the different seeds studied. This self-digestion is made possible because of the fact that much more ptyalin exists in the endosperm cells than in the scutellum, and action of this enzyme may cause starch hydrolysis without aid of the embryo. Bruschi's conclusions also indicate that vitality is possessed by the aleurone cells. In like manner Stoward (19), working with barley, maize, and castor beans, concludes that the pure endosperm tissue of both barley and maize is capable of evolving  $\text{CO}_2$  through the respiratory process. These investigators also attribute a part of the endosperm respiration to the aleurone layer.

All previous work as above noted has been based on studies of seeds which contained both embryo and endosperm, and in order to conduct separate respiration or enzyme tests of these two portions it was necessary to excise the embryos, thus introducing a factor of mechanical injury which might give misleading results.

It has been suggested by Stoward (19) that the greater respiratory intensity of isolated embryos may be attributed in part to a wound stimulus received during their removal from the seed. The work of Tashiro (20) on wheat seed stimulated by injury bears out this statement, for he shows a marked acceleration in respiration due to injury or wound stimulus. The possibility of error due to injury in separating embryos from their endosperms can not be overlooked. The embryoless seed therefore presents a convenient means of eliminating the effect of stimulus to  $\text{CO}_2$  production resulting from wounding. Sufficient embryo-free seeds were found to make possible tests of uninjured seeds containing no embryo structures. To what extent respiration of the normal whole seed is due to the embryo may then be determined by comparison of uninjured embryoless seeds with uninjured normal seeds.<sup>4</sup>

In making these studies a respirometer of simple design was used, consisting of a shell vial 9 cm. deep and 3.5 cm. in diameter, a No. 7 rubber stopper, and a perforated paper cup suspended from the stopper by means of a copper wire. The wire and cup were coated with paraffin in order to prevent any electrolytic effect from the copper wire as well as to render the cup impervious to water.

Five cubic centimeters of  $\text{CO}_2$  free saturated barium hydroxide solution is introduced into the shell vial, which is then quickly stop-

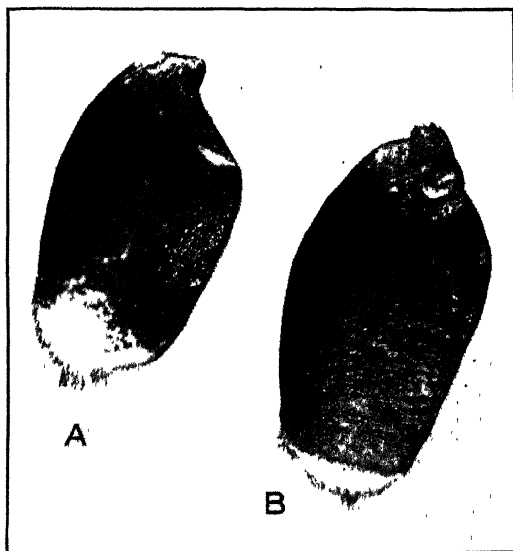


FIG. 2.—Profiles of seeds: A, lacking embryo and B, normal seed. In A the depression due to lack of embryo is very evident

<sup>4</sup> THORNTON, B. J. FACTORS CAUSING LOW GERMINATION IN SORGHUM SEED. 1927. [Unpublished, thesis, Colorado Agr. Col.]

pered. A weighed amount of seed, presoaked for 24 hours, in accordance with Braun's (5) method of seed treatment and then sterilized for two minutes in a 50 per cent alcoholic solution of mercuric chloride, strength 2:1,000, is then placed in the seed cup. The cup and seed are quickly transferred to the shell by substituting the stopper bearing the cup for the stopper which closes the shell. All respiration tests were run for six days at a constant temperature of 20° C., the optimum recommended for the germination of wheat seeds by the Association of Official Seed Analysts of North America (22). At the end of this time the stoppers bearing the seed cups were quickly removed and exchanged for other stoppers. The amount of CO<sub>2</sub> evolved by the seed was determined by titration of the barium hydroxide with N/10 hydrochloric acid and sodium hydroxide. Thymosulphonthalein and tetrabromphenolsulphonthalein were used for indicators as advocated by Harter and Weimer (12). Care was

taken that all apparatus be kept sterile to insure against the production of CO<sub>2</sub> by microorganisms. In each respiration test made, normal seeds and embryoless seeds from the same lot were used, and all seeds both normal and abnormal were carefully examined with a Hasting triplet 7× lens to reduce to a minimum discrepancies arising from the use of injured seed. In order that the results might be considered comparable, the apparatus and methods described above were used in all of the respiration tests.

An average of the results obtained from respiration tests of entire

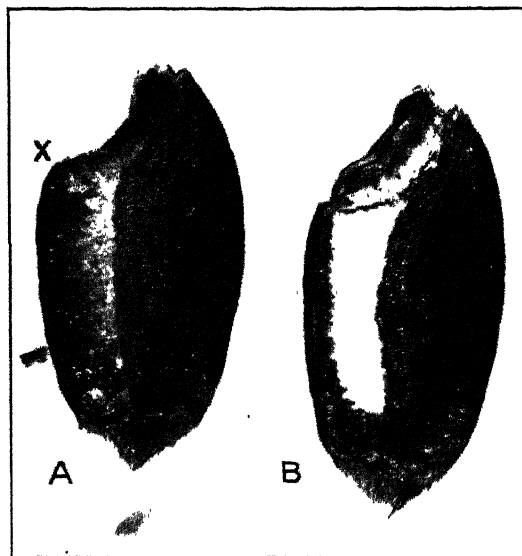


FIG. 3.—Structural differences in longitudinal sections of A, abnormal, and B, normal caryopses of wheat. All embryo tissue is lacking in A, the embryoless seed. The region designated x on this figure corresponds to the region designated x on Figure 4

normal wheat seeds shows a liberation of 26.55 mgm. of CO<sub>2</sub> per gram of seed in six days, as compared with 22.09 mgm. of CO<sub>2</sub> evolved per gram of abnormal seed. These data indicate that CO<sub>2</sub> is liberated by embryo-lacking seed under conditions favorable to germination. There being no embryo present, this activity can be attributed to the endosperm only. From the above figures it would appear that the embryo is responsible for approximately one-sixth of the total CO<sub>2</sub> given off by a respiring wheat seed. It is to be remembered, however, that by weight the embryo represents but 3 per cent of the normal seed, and yet, weight for weight, respire about six times as much as the endosperm. The interesting fact is that the experiment was conducted in a way to obviate CO<sub>2</sub> evolution due to injury or to microorganisms, and shows that uninjured endosperm respire and in a measure not to be inferred from previous studies.

It is generally believed that all respiration in plants and animals is coexistent with enzyme action. According to Bailey and Gurjar (4), that structure of the seed which is the center of respiration gives evidence of the greatest catalase activity. Crocker and Harrington (10), Appleman (2, 3), Morinaga (16), and Choate (9) are among those who have found a rather close correlation between respiratory action and catalase activity. Osterhout (17) states that the cessation of the respiratory process is a test for life but that its continuance can not be accepted as a criterion, since  $\text{CO}_2$  evolution often continues in an organism which has been killed in certain ways.

The study of the respiration of embryo-free seed therefore was accompanied by tests for the activity of amylase as well as catalase. Small amounts of reducing sugars indicating the presence of amylase were found in the imbibed embryoless seeds after they were kept for six days under conditions suitable for germination.

In testing for catalase, the simplified Bunzel apparatus was employed. Appleman's (1) methods were used in conducting the tests. The tissue was ground in a mortar with a small amount of sand and an excess of calcium carbonate. In every case 0.025 gm. of this powdered material was placed in the catalase tube with 5 c. c. of a commercial product, Dioxigen, which had previously been neutralized with N/10 sodium hydroxide. Readings were taken at the end of five minutes.

The results of catalase tests with whole, normal seeds showed an average of 0.17 c. c. of oxygen liberated during a five-minute period for every 0.025 gm. of material used, whereas in the case of embryo-lacking seed 0.12 c. c. of oxygen were evolved per 0.025 gm. of powdered material in the same length of time. These figures indicate that as a result of the catalase activity of a wheat seed 0.12 c. c. of oxygen is liberated by the endosperm while but 0.05 c. c. is evolved by the embryo. This enzyme activity of the endosperm might be inferred from the respiration data given above even though all embryo structures are absent.

#### STRUCTURE OF EMBRYO-FREE SEED

It is generally believed that enzyme secretion as well as the respiratory process is localized in the epithelial layer of the scutellum. In view of the foregoing data on respiration and enzyme activity, a morphological study of seeds in which the embryo has failed to develop might be expected to show a trace of embryo tissue, or at least some epithelial cells. Many sections of such abnormal seeds were made, but no indication of any embryo development was observed. Figure 4 shows a photomicrograph of a longitudinal section of an abnormal wheat seed; the lack of embryo structures in this seed is quite apparent upon comparison with a section of a normal wheat seed as illustrated by Percival (18). No trace of the epithelial layers of the scutellum is evident and all other embryo tissue is absent. The same figure illustrates the junction of the endosperm and the portion normally occupied by the distal end of the embryo. The coat structures are normal, and as in a seed with an embryo, the aleurone cells over the embryo-deficient portion of the seed are greatly diminished in size. The accompanying illustrations exhibit a marked similarity to those of barley figured by Harlan and Pope (11).

## CONCLUSIONS

Embryoless seeds are of frequent occurrence in wheat. Approximately 0.1 per cent of the lots examined manifest this abnormality. Although the endosperm is normally developed, no trace of embryo tissue is found in microsection.

While these seeds can not grow they do respire when placed under conditions favorable to germination and they also exhibit enzyme activity.

It has been found that wheat seeds devoid of embryos respire 22.09 mgm. of  $\text{CO}_2$  per gram of seed in six days, while normal seeds give off 26.55 mgm. of  $\text{CO}_2$  in the same time. It is evident from these figures that the endosperm contributes largely to the respiration of whole normal seeds. The embryo constitutes but approximately 3 per cent of the entire seed by weight. On a weight basis,

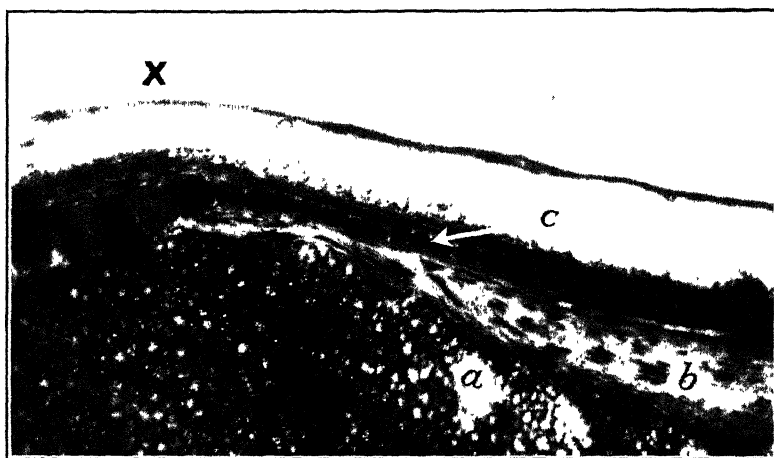


FIG. 4.—Photomicrograph of a section from an abnormal wheat seed taken in the region where the embryo would normally develop; *a*, Amylaceous cells of the endosperm; *b*, collapsed cells apparently of the endosperm tissue; *c*, aleurone layer (note the change in shape of the cells at the point where the aleurone layer of the endosperm joins with that of the embryo cavity; note also that no epithelial layer is present). The region designated *x* in this figure corresponds with the region designated *x* in Figure 3

therefore the respiratory activity of the embryo is decidedly greater than that of the endosperm.

Brief tests show some starch conversion in the embryo-lacking seed after six days in the germination chamber.

Further study shows a catalase activity in these seeds equivalent to 0.12 c. c. oxygen production in five minutes as compared to 0.17 c. c. oxygen produced in the same length of time by equal weights of normal seeds.

Various investigators have studied the respiration of the embryo in its relation to the entire seed, but always after excising the embryo. The occurrence of embryo-lacking seeds offers a solution to the questioned effect of mechanical injury in studying respiration of the embryo as compared to the endosperm.



## LITERATURE CITED

- (1) APPLEMAN, C. O.  
1910. SOME OBSERVATIONS ON CATALASE. *Bot. Gaz.* 50: 182-192, illus.
- (2) ———  
1916. RELATION OF OXIDASES AND CATALASE TO RESPIRATION IN PLANTS. *Amer. Jour. Bot.* 3: 223-233.
- (3) ———  
1918. RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN. *Amer. Jour. Bot.* 5: 207-209, illus.
- (4) BAILEY, C. H., and GURJAR, A. M.  
1918. RESPIRATION OF STORED WHEAT. *Jour. Agr. Research* 12: 685-713, illus.
- (5) BRAUN, H.  
1919. PRESOAKING AS A MEANS OF PREVENTING SEED INJURY DUE TO DISINFECTANTS AND OF INCREASING GERMICIDAL EFFICIENCY. *Science* (n. s.) 49: 544-545.
- (6) BROWN, H. T., and MORRIS, G. H.  
1890. RESEARCHES ON THE GERMINATION OF SOME OF THE GRAMINEÆ. PART I. *Jour. Chem. Soc. [London]* 57: 458-528, illus.
- (7) BRUSCHI, D.  
1908. RESEARCHES ON THE VITALITY AND SELF-DIGESTION OF THE ENDOSPERM OF SOME GRAMINACEÆ. *Ann. Bot. [London]* 22: [449]-463.
- (8) BURLAKOW, G.  
1897. UEBER ATHMUNG DES KEIMES DES WEIZENS, TRITICUM VULGARE. *Arb. Naturf. Gesell. K. Univ. Charkow* 31 (Beil.): I-XV. [In Russian. Abstract in *Bot. Centbl.* 74: 323-324, 1898.]
- (9) CHOATE, H. A.  
1921. CHEMICAL CHANGES IN WHEAT DURING GERMINATION. *Bot. Gaz.* 71: 409-425, illus.
- (10) CROCKER, W., and HARRINGTON, G. T.  
1918. CATALASE AND OXIDASE CONTENT OF SEEDS IN RELATION TO THEIR DORMANCY, AGE, VITALITY, AND RESPIRATION. *Jour. Agr. Research* 15: 137-174, illus.
- (11) HARLAN, H. V., and POPE, M. N.  
1925. SOME CASES OF APPARENT SINGLE FERTILIZATION IN BARLEY. *Amer. Jour. Bot.* 12: 50-53, illus.
- (12) HARTER, L. L., and WEIMER, J. L.  
1921. RESPIRATION OF SWEET POTATO STORAGE-ROT FUNGI WHEN GROWN ON A NUTRIENT SOLUTION. *Jour. Agr. Research* 21: 211-226, illus.
- (13) KARCHEVSKI.  
1901. [INFLUENCE OF THE FLUCTUATION OF TEMPERATURE ON THE RESPIRATION OF SEEDS AND EMBRYOS OF WHEAT.] *Warsaw Univ. Mem.* 9, 114 p. [In Russian. Abstract in *U. S. Dept. Agr. Expt. Sta. Rec.* 14: 839, 1903.]
- (14) KOLKOWITZ, R.  
1901. UEBER DIE ATHMUNG RUHENDER SAMEN. *Ber. Deut. Bot. Gesell.* 19: 285-287.
- (15) MANN, A., and HARLAN, H. V.  
1915. MORPHOLOGY OF THE BARLEY GRAIN WITH REFERENCE TO ITS ENZYM-SECRETING AREAS. *U. S. Dept. Agr. Bul.* 183, 32 p., illus.
- (16) MORINAGA, T.  
1925. CATALASE ACTIVITY AND THE AEROBIC AND ANAEROBIC GERMINATION OF RICE. *Bot. Gaz.* 79: 73-84.
- (17) OSTERHOUT, W. J. V.  
[1924]. THE NATURE OF LIFE. 117 p. New York.
- (18) PERCIVAL, J.  
[1921]. THE WHEAT PLANT; A MONOGRAPH. 463 p., illus. London.
- (19) STOWARD, F.  
1908. ON ENDOSPERMIC RESPIRATION IN CERTAIN SEEDS. *Ann. Bot. [London]* 22: [415]-448.
- (20) TASHIRO, S.  
[1917]. A CHEMICAL SIGN OF LIFE. 142 p., illus. Chicago, Ill.
- (21) TORREY, J. C.  
1902. CYTOLOGICAL CHANGES ACCOMPANYING THE SECRETION OF DIASTASE. *Bul. Torrey Bot. Club* 29: 421-435, illus.
- (22) UNITED STATES DEPARTMENT OF AGRICULTURE.  
1927. RULES FOR SEED TESTING. *U. S. Dept. Agr. Circ.* 406, 13 p.



# A SIMPLE METHOD FOR LIFE-HISTORY STUDIES OF ROOT-FEEDING ARTHROPODS<sup>1</sup>

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## INTRODUCTION

Detailed studies of the life histories of insects which spend all or part of their life cycle feeding upon the underground parts of plants have been exceedingly difficult because of the inaccessibility of the specimens to ready observation. The eggs of most insects which pass their immature stages underground are usually deposited in the soil on or near their host and are generally difficult to find. After the eggs have hatched the immature forms are not readily located and a search for them, no matter how carefully conducted, usually results in the accidental destruction of a large percentage of the specimens.

These difficulties were constantly encountered in an investigation involving a study of the larvae of the striped cucumber beetle (*Diabrotica vittata* Fab.). In these experiments it was necessary to use large numbers of the larvae and to be able to determine their condition at any time, as well as to be assured of a sufficient supply of specimens at any required stage in the life cycle. It was impossible to obtain these conditions when the beetles were permitted to reproduce in the usual manner.

After some experimenting, a method for the collection and incubation of eggs, the maturing of larvae, and the production of adults, all under immediately accessible conditions, was perfected and found to be very advantageous for the purpose of the investigation. Some additional study, in which various other insects and arthropods were used, has demonstrated that the method is easily adaptable to the investigation of the life histories of most of the forms which spend at least a part of their life in the soil. This article, however, will deal only with the method as it was employed in the study of the development of the striped cucumber beetle.

## CAGE FOR COLLECTION OF EGGS

Cylindrical cages of 12-mesh wire screen cloth 10 cm. long and 6 cm. in diameter were used for confining male and female beetles. These were made by soldering to one end of each cylinder the bottom cut off of a tin salve box; the upper part of the box, over which the cover fitted, was soldered to the other end of the cylinder. (Fig. 1.) The cover of the salve box was used to close the cage. The cages were laid upon moist blotting paper and pressed into a quantity of soft soil heaped up in pie tins. Green blotting paper to contrast with the orange color of the eggs was used. By placing the cages in

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pie tins they could be readily moved to any desired location. Food could be easily placed in the cages by simply removing the salve-box cover from the end of the cage.

The beetles oviposit through the wire screen cloth upon the blotting paper. The eggs are then exposed by lifting the cage from the blotting paper. They adhere to the moist blotting paper but are easily removed when the paper dries. The drying should be done slowly and carefully in order to prevent the collapse of the eggs. It is feasible, but not so expeditious, to dispense with the blotting paper, since the screen walls of the cage will restrain the beetles from placing their eggs in the soil. In such cases the eggs will be found on the soil adjacent to the walls of the cage. By varying the number of females in a cage, it is possible to determine and collect the number of eggs laid by one or by a number of females.



FIG. 1.—Oviposition cage for the striped cucumber beetle. Beetles confined in the cage oviposit through the screen wire on blotting paper laid over the surface of the soil

#### CAGE ARRANGEMENT FOR INCUBATION OF EGGS

Cells made from short pieces of glass tubing, one end being stopped with plaster of Paris, were used for the incubation of eggs. (Fig. 2.) Glass cells ranging in height from 1 cm. to 3 cm. and in diameter from 1.3 cm. to 10 cm. were tested. Cells 3 cm. in diameter and 2.3 cm. in height, however, gave the best results. Various thicknesses of plaster of Paris in the cells were tested, and a thickness of 0.3 cm. proved to be the most satisfactory. A convenient method for obtaining uniformity of depth of the plaster of Paris in the cell consists of first mixing the plaster with water to the consistency of fresh cream and then pouring the mixture to a depth of 0.3 cm. into paraffined pie tins. The sections of glass tubing are then placed on end in the mixture. Melting the paraffin after the plaster of Paris has set releases the pie tin, and the plaster of Paris can be removed readily

from each cell. It is necessary that all paraffin adhering to the bottom of the cells be removed. If it is allowed to remain, it will hinder the passage of the water into the cells. To provide a ready method of maintaining a fairly constant moisture content in the cells, they are placed open end upward in soil in pie tins to a depth equal to that of the height of the plaster of Paris in the cells. (Fig. 2.) A number of the cells may be placed in the same pie tin, but care should be taken to see that there is a small area of soil space between each cell. The moisture supply is maintained by adding water at intervals to the soil in the pans. It should be only sufficient to keep the surface of the plaster moist. The presence of free water on the

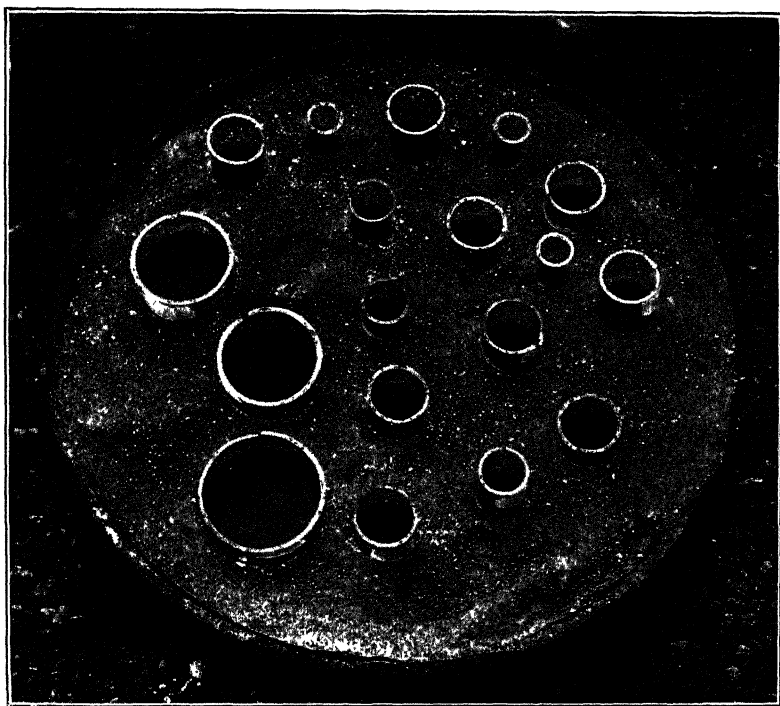


FIG. 2.—Incubation cells used in studies on the striped cucumber beetle. Sections of glass tubing filled to a depth of 3 mm. with plaster of Paris, pressed into moist soil, and the soil between the cells covered with sand

plaster of Paris will cause the death of the embryos. Covering the soil with sand to a depth of about 1 cm. between the incubation cells will aid materially in reducing soil evaporation. Placing the incubation cells in pie tins renders them easily portable.

The larvae and eggs are made easily visible by painting the surface of the plaster with black india ink.

Under this method of treatment the eggs were completely accessible throughout the incubation period. The viability of the eggs as well as the exact moment of emergence can be determined, and the behavior of the larvae upon hatching and the effect of temperature and humidity upon the eggs can be noted. Many eggs, the number

depending upon size, can be placed in one cell for incubation. It is necessary only that the eggs be in contact with the moist plaster of Paris.

#### CAGE ARRANGEMENT FOR REARING LARVAE

Petri dishes containing plaster of Paris were used in rearing the larvae. (Fig. 3.) These were prepared as follows: Plaster of Paris mixed with water to the consistency of fresh cream was poured into the dishes to the depth of about 6 mm. and permitted to set. Squash plants in the seedling stage were removed from the soil in which they had grown, practically all of the soil washed from the roots, and the plants placed in the Petri dishes with the roots spread out upon the

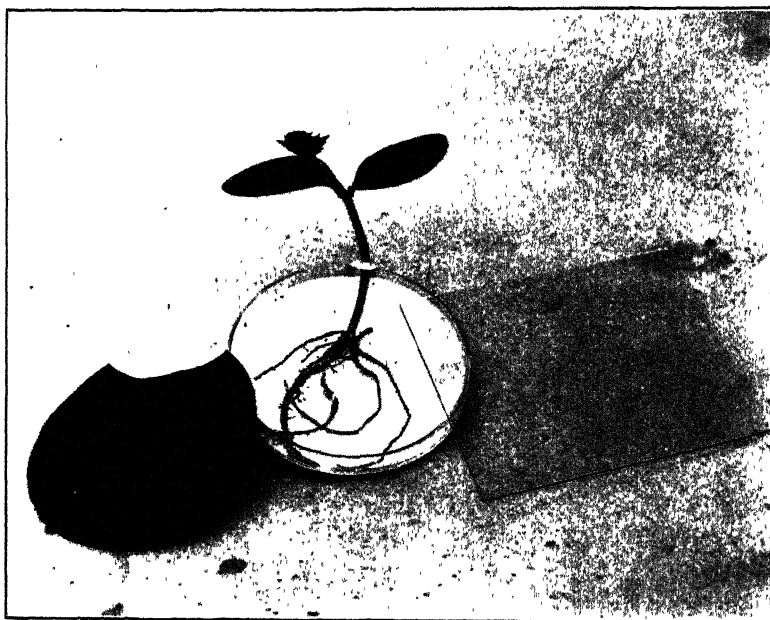


FIG. 3.—Details of Petri-dish rearing cage for larvae of the striped cucumber beetle. The blotting paper shown at the left was laid over the roots and the whole saturated with the nutrient solution. The glass cover shown at the right was then placed over the dish.

plaster of Paris and the leaves extending outward over the sides of the dishes. Pieces of blotting paper cut to fit closely to the sides of the dishes, except where the stem of the plant extended from the dish, were then laid over the roots and the whole saturated with a nutrient solution. To assist in the retention of the larvae and to avoid rapid evaporation of the solution, pieces of glass were laid over the dishes, a small opening being left at the plant stem to permit aeration. It is quite feasible to use larger Petri dishes for larger arthropods and larger plants. A flat dish with a lip could be used conveniently, since the lip would provide an opening for the stem of the plant. The part of the opening not occupied by the stem could be filled with raw cotton.

## FORMULA FOR NUTRIENT SOLUTION

The nutrient solution used was one suggested by W. E. Tottingham, professor of agricultural chemistry at the University of Wisconsin, and was of the following formula:

	Grams		Grams
Sodium nitrate-----	2.0	Calcium sulphate (hydrated)----	0.2
Disodium phosphate (hydrated)---	.2	Magnesium sulphate (hydrated)---	.2
Monosodium phosphate (hydrated)-----	.1	Ferric citrate (made by heating a 1 per cent solution, 1 gm. to 100 c. c. of water. Use 5 c. c. per liter)-----	.05
Potassium chloride-----	.4		
Water to make 1 liter.			

This is a general-purpose formula. Legumes as well as cucurbits and grasses grew well in the Petri dishes when a solution made by this formula was used.

## MANIPULATION OF LARVAL CAGES

When the solution is used, it is necessary that an excess be avoided, since free liquid about the roots will cause the death of both plants and larvae. The insect and plant thrive best when the plaster of Paris and the blotting paper are merely moist. The quantity of solution necessary to maintain this condition varies with the temperature and humidity of the surrounding atmosphere, but it can be easily determined by noting the condition of the blotting paper. The addition of about 4 c. c. of the solution to each dish daily was usually sufficient.

## TRANSPLANTING PLANTS

In these experiments plants of Hubbard squash were used because of their large size and strong root system. To obtain the best results it was necessary to germinate the plants in soil and to remove them to the Petri dishes while they were still in the cotyledon stage and before the fibrous roots were formed. In experiments with cucurbits and legumes it was found that the plants were not able to utilize the solution except through the water roots. Old plants which had lost their water roots either died or remained wilted until new water roots were formed.

## TRANSFERRING LARVAE

The newly emerged larvae are easily injured and should be handled with great care. It was found that they could be transferred without injury from the incubation cells to the Petri dishes by barely touching them with the flattened end of a piece of wire which had been slightly moistened with water. The larvae were immediately held to the wire by the surface tension of the water; this evaporated quickly and released them at any desired place in the Petri dish at which the wire was held.

## OBSERVING LARVAE

To observe the larvae at any time it is necessary only to remove the cover glass and blotting paper. This is one of the greatest advantages of the method since the insects are always readily accessible. In addition, a great many of them may be reared in a small space. (Fig. 4.)

## CONTAMINATION OF THE CAGES WITH GREEN ALGAE

Green algae grew readily in the nutrient solution and caused some trouble. When the alga becomes abundant, it forms a coating over the paper and the plaster, inhibiting the free absorption of the solution. When this condition exists, the plants do not grow well, and therefore care should be taken to prevent the algae from contaminating the stock solution or the Petri dishes. Autoclaving the solution and changing the blotting papers occasionally will serve to hold the algae in check. Petri dishes used as cages may be salvaged for further use by subjecting them to dry heat at a temperature of about 500° F. for one-half hour. The plaster of Paris contracts under this treatment and is readily removed.

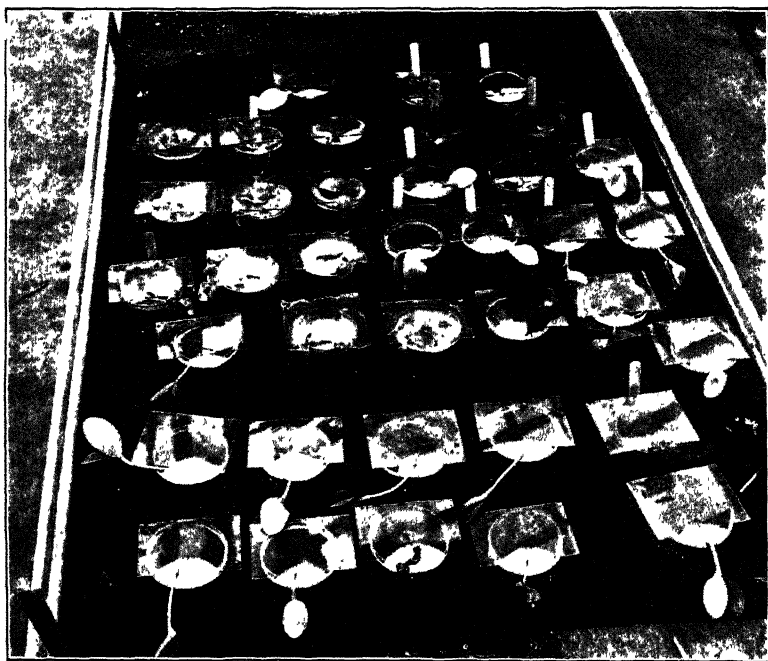


FIG. 4.—Series of rearing cages used in life-history studies of the striped cucumber beetle. This series occupied about 5 linear feet of a greenhouse bench and was sufficient for rearing about 300 larvae

## CAGES FOR PUPATION

When the larvae reach the prepupal stage, as indicated by restlessness and cessation of feeding, they can be removed to small pasteboard pill boxes containing a small quantity of moist soil.

## LENGTH OF LIFE CYCLE

The conditions imposed by the method herein described may accelerate or retard the development of some species of arthropods, and for accurate life-history studies it would be well to run a check by rearing some specimens under natural conditions in soil. It was found that the striped cucumber beetle completed a life cycle, under the conditions imposed by this method, in an average of 34 days.



Beetles permitted to reproduce on potted squash plants required an average of 37 days to complete the life cycle. The time required for the completion of each stage under each method was almost exactly proportional to the average total time required under each method. This condition may not hold with all other arthropods, and the duration of stages in the life history of those reared in plaster of Paris cages and of those reared under natural conditions should be considered comparable only where tests have shown them to be comparable.

#### ADVANTAGES OF THE PLASTER OF PARIS CAGES

1. Individuals or groups are immediately accessible for any purpose throughout their life cycle.
2. Phytopathological studies involving arthropods are greatly facilitated.
3. Individuals or groups may be easily segregated throughout the life cycle. Pedigreed specimens may be reared if desired.



# A STUDY OF THE APPARENT VISCOSITY OF MILK AS INFLUENCED BY SOME PHYSICAL FACTORS<sup>1</sup>

By GEORGE MONROE BATEMAN<sup>2</sup> and PAUL FRANCIS SHARP, *Department of Dairy Industry, New York State College of Agriculture, Cornell University*

## INTRODUCTION

For many years numerous studies of the viscosity of whole milk and cream have been carried out. The methods used by most of the investigators have been very empirical. No careful investigation of the viscosity of milk and cream at various rates of shear has been reported in the literature.

A great variety of conditions and methods were used to determine the viscosity of milk. This makes it impossible to compare the work of one experimenter with that of another, except in a general way, since one does not know whether or not their results are independent of the dimensions and peculiarities of the apparatus they used. The correction for kinetic energy or inertia has been ignored. Some investigators have made a comparison of the relative rates of flow of water and milk by means of an ordinary pipette, and have apparently assumed that this method gave a measure of viscous flow, since they have designated their results "relative viscosity." In most of the work the standard of comparison was water, and frequently no attempt was made to run the determinations at exactly the calibration temperature. The importance of careful temperature regulation is paramount when it is realized that the fluidity of water changes from 1 to 3 per cent for a change of 1° C. Most of the investigations have been carried out at widely varying temperatures, which increases the difficulty of the problem of weighing the relative value of the results. Dahlberg and Hening (13)<sup>3</sup> worked in a refrigerator where the temperature was kept between 3° and 4° C. Kobler (20) carried out most of his experiments within a temperature range of 15° to 18° C., while Taylor (30) worked at 20° C. Cavazzani (12) studied the viscosity of milk at 37°. A number of workers have ignored the difference in density between the calibration liquid, water, and the milk or cream whose viscosity was being determined. This would be a source of error in an Ostwald viscometer where the driving force is entirely due to the hydrostatic head of the contained liquid. The omission of such data as fat content, total solids, previous history of the sample, etc., also complicates the difficulty of making a quantitative comparison and estimation of the published work on the viscosity of milk and cream.

Another subject which has been largely ignored in the past is, how the apparent viscosity of skim milk is affected by different physical factors. In almost all the work done only the viscosity of whole

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<sup>2</sup> Abstracted from a portion of a thesis presented by George Monroe Bateman to the graduate school of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1927.

<sup>3</sup> Reference is made by number (*italic*) to "Literature Cited," p. 672.

milk and the factors that influenced it have been considered. Very little study has been made of skim milk as a separate phase in which changes are taking place along with those (clumping and creaming) in the fat phase. Consequently, very little is known concerning the changes in whole milk which are actually due to the alterations taking place in the skim-milk phase.

No investigator of the viscosity of milk and cream has taken the later concepts of plastic and viscous flow into consideration in his work.

The conditions of flow which a liquid must fulfill when a given volume is forced through a capillary using various pressures to produce the flow, in order that it may be said to follow Poiseuille's law, are as follows:

$$\eta = Pt \text{ (constant)}^4 \quad (1)$$

where  $\eta$  is the viscosity coefficient,  $P$  the pressure, and  $t$  the time of flow. This equation shows that the viscosity of a liquid which follows Poiseuille's law is independent of the pressure producing the flow. The extent to which the flow of milk through a capillary is in agreement with this relationship has never been accurately determined.

Since the conditions under which equation (1) holds experimentally are inconvenient for investigational work, the equation has been modified by correction factors so that the capillary need not be so small and so that high pressures can be used. Bingham and White (8) have used the equation in the following form:

$$\eta = \frac{\pi g r^4 P t}{8 v (l + \lambda)} - \frac{m v \rho}{8 \pi t (l + \lambda)} \quad (2)$$

where  $\eta$  = viscosity coefficient.

$g$  = gravitational constant.

$r$  = radius of capillary in centimeters.

$P$  = pressure in grams per square centimeter.

$t$  = time of flow in seconds.

$v$  = volume of flow in cubic centimeters.

$l$  = length of capillary in centimeters.

$m$  = constant whose value was taken as 1.12.

$\rho$  = density of liquid.

$\lambda$  = a correction to be added to the length of the capillary to correct for the modification of the stream lines at the terminals of the capillary, which cause a loss of pressure in overcoming friction.

The term involving  $m$  is generally called the kinetic energy correction, and, as will be seen from equation (2), it is a subtractive correction to be applied to  $Pt$  to cover a loss of head or pressure rising from the imparting of kinetic energy to the liquid. This correction is usually very small and approaches zero in the case of long, narrow capillaries, or in cases where the value of the pressure,  $P$ , is very small.

<sup>4</sup> This equation holds experimentally only for very long, narrow capillaries or for very small pressures.

## EXPERIMENTAL

## APPARATUS AND GENERAL PROCEDURE

This work was undertaken, first, to make a systematic study of the viscosity of skim milk under varying conditions of treatment; second, to see if some of the changes which take place in the viscosity of whole milk are due to changes associated with the fat or to changes in the skim-milk phase; and third, to investigate the rate of flow of milk through capillary tubes at varying pressures. Practically no work has been done on these phases of the subject.

The methods outlined below are general. The special procedure followed in each phase of the experimental work will be presented, together with the resultant data.

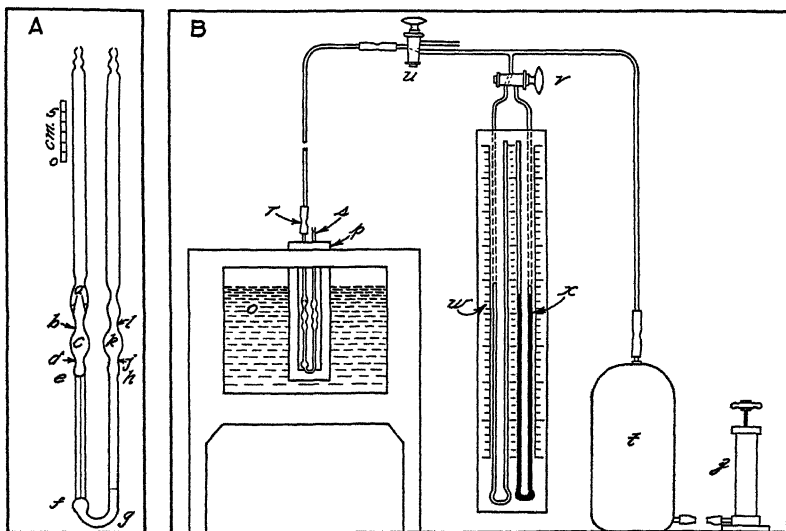


FIG. 1.—A, Important features of the Bingham viscometer; B, apparatus used to apply variable pressure to the viscometer

## MILK USED

The milk used in the experiments was obtained from the Cornell University dairy herd. It was filtered through cotton into sterile bottles, and immediately after milking it was cooled to  $0^{\circ}$  C. to prevent, as far as possible, contamination or decomposition. When skim milk was desired, the fat was removed by centrifuging the properly cooled whole milk in 250 c. c. bottles at about 2,500 revolutions per minute for 30 minutes. The skim milk was carefully removed, filtered into sterile storage bottles, and cooled to about  $0^{\circ}$  C. In all cases dust contamination was guarded against.

Since most of the milk used in the experiments was obtained under conditions that would insure a low bacterial content and freedom from dirt, no attempt was made to get representative samples. The milk was taken from single cows at various stages of milking. Hence in many cases the ratio between total solids and milk fat was not always representative of normal whole milk.

The fat content was determined according to the Roesse-Gottlieb method as modified by Mojonnier (Mojonnier and Troy (22)). The milk solids determinations were also carried out with the Mojonnier milk solids testing apparatus.

#### APPARATUS

Figure 1, A, gives the details of the Bingham (6) viscometer, and Figure 1, B, shows a view of the whole set-up of the apparatus used. *t* represents a galvanized tank of about 25 liters capacity, with an automobile tire valve soldered to it to serve as an intake valve. Air was compressed into the tank by means of a hand pump *z*. Glass tubing connected the tank *t* to the manometers (*x* mercury and *w* water), and to the viscometer at *r*. At low pressures the water manometer, *w*, was used, while at higher ones the mercury manometer, *x*, could be utilized by turning the three-way stopcock, *v*, halfway around.

The viscometer was connected to the pressure line by means of a three-way stopcock, *u*. This stopcock when turned in one direction connected the viscometer to the laboratory air, and when turned in the other connected it to the pressure line. The viscometer was held rigidly in a solid wooden frame, *p*, which fitted in a slot in the water bath, *o*, so that it could be replaced in the same position after removal from the water bath for cleaning.

The temperature of the water bath was kept constant  $\pm 0.01^\circ \text{C}$ . by means of an automatic mercury thermo regulator. All determinations were carried out at  $25^\circ \text{C}$ . A Beckmann thermometer was immersed in the water bath to check the temperature at frequent intervals.

#### CALIBRATION

The Bingham viscometer used in this work was purchased from Eimer and Amend, and the capillary was specified as 500, according to the requirements set by Bingham (6). With such a capillary fluidities from 0.5 to 500 can be measured. This viscometer, if properly made, has all the desirable features of the Ostwald viscometer, and practically none of its objectionable ones. From the diagram (fig. 1, A) it will be noted that the viscometer is constructed so as to reduce the effect of the hydrostatic pressure of the contained liquid to zero. Each arm of the instrument is made as nearly as possible similar to the other in shape and height, and the bulbs have the same volume. The value of the density of the contained liquid need not be known with considerable accuracy. A trap is provided in one arm to make it possible to work with exactly the same volume of liquid at all temperatures.

With the Bingham viscometer the force driving the liquid through the capillary is entirely due to the pressure that is being exerted on the system, providing the viscometer is constructed so that the hydrostatic head of the contained liquid is equal to zero.

Inasmuch as it would be too difficult and laborious to construct a viscometer for ordinary work where all of the dimensions could be accurately determined by direct measurement, it is a common practice to calibrate the instrument with some suitable standard liquid. For a given instrument it will be noted that certain of the factors in equation (2) remain constant in all of the determinations and that these may be presented by *C* and *C'*. Hence

$$C = \frac{\pi g r^3}{8 l v} \quad (3)$$

and for the kinetic energy correction factor,

$$C' = \frac{m v}{8 l \pi} \quad (4)$$

Equation (2) now becomes:

$$\eta = C P t - C' \rho / t \quad (5)$$

where  $C$  and  $C'$  are constants, which may be determined.

In this work the value of  $C'$  was found by direct measurement. The volume,  $v$ , of the bulb,  $c$  (fig. 1, A), between the marks  $b$  and  $d$  was found to be equal to 4.0 c. c. and the length of the capillary 10 cm. Hence

$$C' = \frac{1.12 \times 4.0}{8 \times 3.1416 \times 10} = 0.01784$$

To determine the viscometer constant  $C$ , it was first necessary to solve equation (5), which gives:

$$C = \frac{\eta t + C' \rho}{P t^2} \quad (6)$$

A number of viscosity runs were made with dust-free, freshly distilled water at 25° C. to determine the time of flow at known varying pressures for each limb of the viscometer. The mean value of the viscometer constant was found to be equal to  $1.319 \times 10^{-7}$ .

For the viscometer used in this work, equation (5) now becomes:

$$\eta = 1.319 \times 10^{-7} P t - 0.01784 \rho / t \quad (7)$$

where  $\eta$  is the viscosity in centipoise.

The flow periods for each arm were found to be approximately the same, since no variation was greater than one-fifth of a second. Hence the correction for the hydrostatic pressure of the liquid in the viscometer was unnecessary with this instrument.

In the course of these experiments we tried to maintain a constant room temperature. The effect of slight variations in the temperature of the room and the corresponding effect on the pressure in the tank was one of the greatest sources of error with which the authors had to contend. This error could have been greatly reduced by placing the pressure tank in the water bath. The change in pressure due to slight changes in temperature during each determination was usually so small that it could not be detected on the manometers. Also it was impossible to measure time periods with greater accuracy than one-fifth of a second with an ordinary stop watch. In order to minimize the above sources of error as much as possible, the efflux periods throughout these experiments were largely maintained between 100 and 1,000 seconds. The stop watch was always kept wound to about the same tension, and at frequent intervals it was checked for accuracy by means of a standard chronometer.

THE INFLUENCE OF PRESSURE, AS A VARIANT, ON THE APPARENT VISCOSITY OF SKIM MILK, SKIM COLOSTRUM, RAW, PASTEURIZED, AND HOMOGENIZED WHOLE MILK, AND EVAPORATED SKIM MILK

It is rather surprising that most of the investigators have overlooked the effect of the rate of shear on the viscosity of milk and cream.

Galdi (15) attempted to determine the viscosity of a large number of liquids, including milk, by means of a Hirsch-Beck apparatus. His apparatus really consisted of a modified Ostwald viscometer connected to a source of pressure. He determined the relative viscosity of milk at a number of pressures, but his data show comparatively large inconsistent variations. His results vary between 1.57 and 1.67 and are of little value in showing the effect of the rate of shear on the viscosity of milk.

The only other reference covering the relation between the viscosity coefficient of milk and the pressure that could be found in the literature was a statement by Rothlin (26) that mixtures of emulsoids and suspensions such as milk, defibrinated and normal blood, show deviations from Poiseuille's law at low rates of shear. Rothlin (25) presented no results on milk, however, even in his more extensive paper.

If Rothlin's observations are correct the question as to absolute values for the viscosity of milk arises. If a physical "constant" shows variation with the conditions it is being subjected to when determined, it is obvious that these conditions should be specified. This is especially true where changes in the structure of the constituents caused by various kinds of treatment are being considered.

These series of experiments constitute a study of the influence of varying the rate of shear on the apparent viscosity of skim milk, skim colostrum, raw, Pasteurized, and homogenized whole milk, and evaporated skim milk. The viscometer was filled accurately with a definite volume of milk, in such a manner that the aliquot of the test milk was not passed through the capillary in the filling process. The viscometer was cleaned and dried for each individual determination.

The data which follow were selected from a number of experiments as being typical of the effect of the rate of shear on the viscosity of milk.

TABLE 1.—*Effect of varying pressure on the viscosity of skim milk*

Sample No.	Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
1-----	1,146.3	96.4	1.438
	982.0	112.6	1.442
	681.6	162.7	1.450
	480.3	231.4	1.458
	278.1	401.4	1.468
	150.1	743.4	1.470
2-----	981.2	109.0	1.393
	814.8	131.4	1.398
	682.2	157.2	1.403
	480.3	223.0	1.405
	278.1	385.0	1.408
	981.2	117.2	1.500
3-----	814.8	140.0	1.491
	681.6	168.8	1.506
	480.9	238.6	1.507
	278.1	415.6	1.519
	143.1	808.6	1.516
	71.6	1,612.8	1.516
4-----	981.7	113.3	1.452
	815.7	136.8	1.458
	682.4	163.9	1.464
	481.7	232.3	1.468
	278.7	402.2	1.474
	147.9	760.4	1.481
	71.9	1,579.2	1.496



TABLE 2.—*Effect of varying pressure on the viscosity of fresh skim colostrum*

Number of days after parturition	Pressure gm. per sq. cm.	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
4	1,306.14	104.4	1.781
	979.7	139.3	1.787
	679.3	201.6	1.797
	275.3	498.4	1.806
	132.21	1,051.2	1.831

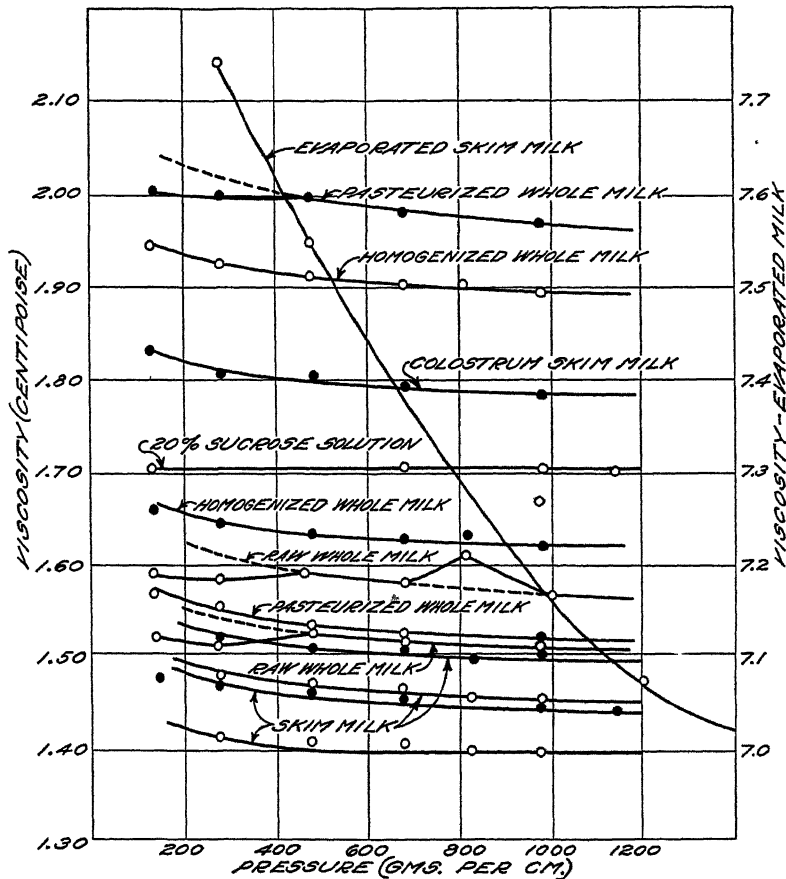


FIG. 2.—Decrease in viscosity of various kinds of milk as shearing force is increased

The viscosity of skim milk and skim colostrum at varying pressures was studied first. The results are given in Tables 1 and 2, and graphically in Figure 2. From these data it can be seen that the viscosity coefficient of skim milk is not constant but varies with the pressure. When the pressure is increased the apparent viscosity is slightly decreased. Skim colostrum (four days after parturition) also gave pressure-viscosity curves which resembled those of skim milk.

TABLE 3.—*Effect of varying pressure on the viscosity of raw whole milk*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
4	3.58	12.86	1,306.9	91.9	1.548
			978.3	122.7	1.567
			812.1	151.9	1.613
			679.4	177.7	1.581
			477.5	254.0	1.591
			275.9	437.0	1.587
			131.8	917.8	1.593

TABLE 4.—*Effect of varying pressure on the viscosity of Pasteurized whole milk*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
8	4.75	14.18	980.4	153.2	1.970
			680.1	221.7	1.981
			481.5	314.9	1.999
			277.2	548.5	2.001
			145.85	1,051.9	2.023
			1,306.9	89.6	1.523
6	3.83	12.82	979.7	118.2	1.511
			678.8	170.4	1.514
			478.8	242.6	1.524
			275.9	418.2	1.514
			138.0	834.6	1.522
			1,308.5	88.4	1.511
1	-----	-----	980.6	118.7	1.517
			680.9	171.2	1.526
			478.9	244.2	1.535
			276.7	427.4	1.555
			138.2	863.0	1.571

Experiments with whole milk are given in Tables 3 and 4, and are graphically represented in Figure 2. Much difficulty was experienced in attempting to determine the apparent viscosity of raw or Pasteurized whole milk. At the lower pressures it was practically impossible to obtain concordant results. It will be noted that the curves for raw and Pasteurized whole milk shown in Figure 2 are not all consistent, and that the apparent viscosity values at low pressures are much less than the theoretical (extrapolated) ones, shown by the broken lines. These discrepancies are due to the fact that at low pressures the time periods are sufficiently long to allow part of the cream to rise to the surface in the viscometer. This means that as the time of flow increases, more of the butter fat will have a chance to rise, and the composition of the milk flowing through the capillary will approach that of skim milk. Hence its apparent viscosity will tend to decrease with the longer time periods.

The results obtained with homogenized whole milk appear to support the above conclusion. In homogenized whole milk the fat globules are not clumped and are broken up into sufficiently finely

divided particles so that they remain suspended throughout the milk. With such a system a viscosity-pressure curve that resembles those of skim milk and skim colostrum can be expected. The results obtained with homogenized whole milk are given in Table 5, and are plotted as curves in Figure 2. The viscosity values obtained with homogenized whole milk were very consistent and reproducible.

TABLE 5.—*Effect of varying pressure on the viscosity of homogenized whole milk*

Number of days aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
1	2.94	11.64	1,307.8	94.8	1.616
			980.6	126.6	1.623
			815.6	152.2	1.626
			680.9	182.5	1.629
			480.3	259.3	1.636
			278.1	450.0	1.647
			136.4	920.0	1.659
			1,306.9	110.6	1.892
1	4.63	14.08	979.7	147.3	1.892
			812.7	178.4	1.902
			679.4	213.2	1.901
			479.7	303.7	1.915
			276.6	529.5	1.928
			134.46	1,059.6	1.947

The sample of evaporated skim milk studied in this series of experiments, showed the greatest variation of viscosity with pressure. A glance at Figure 2 and Table 6 makes this fact evident.

TABLE 6.—*Effect of varying pressure on the viscosity of evaporated skim milk*

Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
Fat	Total solids			
0.72	25.63	1,466.2	366.8	7.089
		980.4	562.0	7.252
		814.2	671.2	7.205
		480.3	1,186.2	7.513
		276.0	2,127.2	7.744

In order to make sure that the value of the capillary constant *C* was correct, and that the results, as obtained, were not due to some peculiarity of the apparatus, a set of runs were performed on a 20 per cent pure sucrose solution. According to Bingham and Jackson (?) this solution should have a viscosity of 1.704 centipoise at 25° C. As will be noted by referring to the data in Table 7 and Figure 2, the viscosity of the solution at all of the pressures was constant and averaged 1.704 centipoise. This shows that the apparatus was apparently not the cause of the variation in the viscosity of milk with pressure.

TABLE 7.—*Effect of varying pressure on the viscosity of a 20 per cent sucrose solution*<sup>1</sup>

Pressure (gms. per sq. cm.).	Average time of flow (seconds)	Viscosity (centipoise) at 25° C.
1,144.9	113.8	1.703
980.6	132.9	1.704
680.9	191.0	1.704
124.7	1,037.2	1.704

<sup>1</sup> Viscosity according to Bingham and Jackson (7) = 1.704.

If milk were a truly viscous liquid, it should give a constant viscosity value with varying pressures, since according to Poiseuille's law the product of the pressure and time for a given set of conditions should equal a constant. This means that the pressure-viscosity curve should be a straight line parallel to the pressure axis. Since milk does not fit the requirements of the law of Poiseuille, but shows a slight variation with pressure, it can not be called a truly viscous liquid. No term which can be used to designate the flow of a liquid which deviates slightly from Poiseuille's law has come into common use. The terms "apparently viscous" and "plastico-viscous" have been used to designate flow of this type. Inasmuch as the viscosity of milk is not independent of the rate of flow, it can be readily seen that all of the conditions of measurement must be taken into consideration. Hatschek (17) says: "If the viscosity of a given sol varies with the shear gradient, single measurements at arbitrary and unknown shear gradients have no theoretical value, least of all as a foundation for hypotheses regarding structure."

It has already been noted that the viscosity of milk is a function of the pressure and decreases as the pressure increases. The assumption which Hatschek and Jane (18) make to account for this variation is that each particle must be surrounded by an adsorbed layer of liquid, which increases its effective volume and moves with it. This layer is assumed to be very labile, and it is more and more reduced in thickness as the rate of flow increases. The thicker the adsorbed layer the greater will be the viscosity. It will be seen that the decrease of viscosity with increased rate of flow will be related to the amount of this adsorbed film displaced, and that at pressures sufficiently high, the viscosity should approach a constant value.

Hess (19) has also offered an explanation for the decrease in viscosity of suspensions with an increase in shearing force. He considered that the suspended particles, due to their size, obstructed the normal telescopic flow of the dispersion medium, causing "dead" spaces immediately behind and ahead of the particles. These so-called dead spaces removed a part of the liquid from the normal telescopic flow of the dispersion medium and consequently increased the effective volume of the particle, and at the same time decreased the effective volume of the dispersion medium. He believed that as the shearing force increased the telescopic layers displaced more of the liquid in the dead spaces, and consequently the viscosity decreased as the shearing force increased.

## THE EFFECT OF REPEATEDLY FORCING MILK THROUGH A CAPILLARY TUBE AT CONSTANT PRESSURE

Kobler (20) studied the effect of repeatedly running milk through a capillary, and of agitation, on the relative viscosity of whole milk. He forced it through a capillary 40 times, and found that the viscosity was materially diminished. For example his first reading was 1.77, his tenth decreased to 1.70, his twentieth to 1.65, while his values from the thirtieth to the fortieth remained at 1.60. However, after the milk was allowed to stand for 14 hours the viscosity of the sample was found to be 1.70. He also showed that he could diminish the viscosity of normal milk from 1.83 to 1.67 by 20 minutes of agitation, and that if he allowed it to stand for 14 hours the viscosity would return almost to the normal value, if the shaking had not been too severe. Kobler assumed that the decrease was due to the breaking up of a rigid structure in the milk which was capable of forming again on standing, provided the agitation had not been too severe. He made no attempt to explain the actual mechanism of this structure. It is probable that this structure, postulated by Kobler, was really a clumping of the fat globules, and that the decrease in viscosity noted was largely brought about by breaking up these clumps.

This set of experiments constitute a study of the effect of repeatedly running skim milk, skim colostrum, raw and homogenized whole milk through a capillary. The effect of protein and fat grouping as factors influencing the constancy of the viscosity was especially considered.

Much difficulty was experienced with raw whole milk, since the cream had a tendency to rise slowly and stick to the sides of the viscometer reservoirs during the progress of experiments. Churning also had to be guarded against. With homogenized raw milk there was no indication of the fat sticking to the viscometer.

The raw whole milk was secured immediately after milking and cooled to 4° C. It was aged at this temperature for a number of hours to allow the fat globules to clump thoroughly. The milk was next gently mixed by pouring it from one vessel to another. A sample was placed in the water bath and allowed to come to a temperature of 25° C. The milk was again mixed very gently; a portion was added to the viscometer, and continuously forced back and forth through the capillary at constant pressure. At the same time, another part of the same sample was examined under the microscope to note the state of aggregation of the fat globules. The milk was introduced into trenches in a blood-counting cell. A cover glass had been previously cemented over the trenches by means of a mixture of vaseline and paraffin, and after the milk was introduced into the trenches the open ends were sealed with the same paraffin mixture. This gave a thin uniform layer of milk. The microscope was adjusted so that the trenches could be examined in a perpendicular position. The degree of clumping of the fat globules and their tendency to rise either as individuals or as aggregates could be followed very closely. This procedure was repeated with the milk after it had been repeatedly forced through the viscometer capillary.

The milk which was to be homogenized was first carefully heated to 50° C., and then forced through the homogenizer at a pressure of over 4,000 pounds. It was immediately cooled to about 4° C. and kept at this temperature until used. Throughout this work all of the homogenized milk was subjected to the above treatment unless otherwise stated.

The results of this series of experiments are summarized in Tables 8 to 11.

TABLE 8.—*Effect of repeated forcing of fresh skim milk through the viscometer capillary*

Sample No.	Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
1-----	682.9	1	1.361
		3	1.361
		5	1.361
		9	1.361
		11	1.363
		13	1.361
		15	1.362
		17	1.361
		19	1.363
		23	1.361
		27	1.361
		29	1.363
		35	1.363
		39	1.361
2-----	682.1	1	1.428
	682.1	3	1.426
	681.5	5	1.428
	678.8	7	1.427
	682.9	9	1.425
	678.8	11	1.428
	682.1	15	1.428
	682.1	19	1.428
	682.1	21	1.428
		2	1.449
3-----	548.3	4	1.449
		6	1.449
		10	1.450
		14	1.449
		16	1.450
		20	1.449
		24	1.449
		30	1.449
		32	1.450

In Table 8 the results obtained with skim milk are given. From these results it is apparent that continuously running skim milk through the capillary at constant pressure had absolutely no effect on the viscosity values, since they remained constant throughout each set of runs.

From the data in Table 9 it will be seen that the viscosity actually increased as the colostrum skim milk was run repeatedly through the capillary. This increase was probably due to a gradual increase in the viscosity of skim milk upon aging, for considerable time elapsed during the experiment. The higher the protein content the greater is the increase of viscosity with age. The deviation from a constant value tends to decrease as the composition approaches that of normal skim milk. The colostrum taken one day after parturition showed the greatest variation. Also it tended to stick to the walls of the viscometer reservoir, which resulted in poor drainage.

TABLE 9.—*Effect of repeated forcing of fresh colostrum skim milk through the viscometer capillary*

Days after parturition	Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
1-----	683.4	1	3.460
		3	3.473
		5	3.485
		7	3.485
		9	3.485
		11	3.486
		13	3.497
2-----	682.9	15	3.503
		1	1.782
		3	1.785
		5	1.789
		7	1.789
		9	1.789
		11	1.786
		13	1.786
		17	1.787
		21	1.787
		23	1.787
		1	1.518
		3	1.522
3-----	684.5	5	1.522
		7	1.525
		9	1.525
		11	1.525
		13	1.525
		15	1.525
		1	1.525
	690.9	3	1.522
		5	1.522
		7	1.525
		9	1.525
		11	1.525
		13	1.525
		15	1.525

TABLE 10.—*Effect of repeated forcing of milk through the viscometer capillary*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
3	6.04	15.43	814.2	1	1.867
				3	1.847
				5	1.837
				7	1.828
				9	1.818
				11	1.816
				17	1.818
				27	1.818
				29	1.816
				1	1.949
				3	1.915
				5	1.900
				7	1.883
30	6.04	15.43	815.4	9	1.874
				11	1.868
				13	1.863
				17	1.857
				21	1.859
				25	1.857
				1	1.640
6	3.58	12.87	814.0	3	1.638
				7	1.634
				11	1.626
				15	1.626
				19	1.625
				25	1.625
				1	1.625

The results obtained with raw whole milk are given in Table 10, and they indicate that the viscosity values decrease to a constant under the conditions of the experiment. With fresh Jersey milk the viscosity decreased from 1.867 to 1.816 centipoise. After aging for

about 30 hours, the effect of repeatedly running the same sample through the capillary was even greater, falling from 1.949 to 1.857. Similar results were obtained with Holstein milk, the viscosity dropping from 1.640 to 1.625.

A microscopic study of the whole milk which had been repeatedly run through the capillary, and that which had not been, showed that the decrease in viscosity, noted above, was correlated with a breaking up of the fat clusters. In Jersey milk especially the majority of the fat globules were initially present as clumps, but after being subjected to the above treatment the microscopic field showed very few clustered groups of fat globules. The portion of the sample which had been run back and forth through the viscometer showed very little tendency to cream after 20 minutes in the microscopic creamer, while the creaming of the control sample which was clumped was very rapid. Another source of decrease of viscosity which should be noted, was the tendency of the fat to stick to the inner walls of the viscometer reservoirs.

It has been noted that repeatedly running normal skim milk through the capillary had no effect on its viscosity, while with normal whole milk a marked decrease resulted, the effect being greater the higher the fat content of the milk. It was also found that the decrease in viscosity of whole milk was correlated with the breaking up of the aggregates of fat globules. In order to check the above conclusions homogenized whole milk was repeatedly run through the capillary at constant pressure. Representative data covering the results of these operations are included in Table 11. The viscosity values obtained with homogenized whole milk are as constant as those with normal skim milk, under the same experimental conditions. Since the fat globules in homogenized whole milk are not clumped, this result would be expected. These experiments show that the decrease in viscosity of normal whole milk due to repeatedly running the same sample through a capillary or to agitation is accounted for by the breaking up of the clumps of fat globules.

TABLE 11.—*Effect of repeated forcing of homogenized whole milk through the viscometer capillary*

Number of hours aged at 4° C.	Composition (percentage by weight) of—		Pressure (gms. per sq. cm.)	Number of times forced through capillary	Viscosity (centipoise) at 25° C.
	Fat	Total solids			
8	3.58	12.87	814.0	1	1.830
				3	1.829
				5	1.830
				7	1.830
				11	1.829
				15	1.830
				23	1.830
6	2.94	11.63	816.7	1	1.586
			816.7	3	1.586
			816.7	5	1.586
			816.7	7	1.587
			816.7	9	1.586
			816.7	15	1.586
			816.7	19	1.586
			815.6	25	1.585
			815.6	29	1.585
			816.7	35	1.586



## THE EFFECT OF HOMOGENIZATION ON THE VISCOSITY OF MILK

The influence of homogenization on the viscosity of whole and of skim milk was considered in this set of experiments. Much work has been done on the viscosity of homogenized cream, while the effect of this treatment in the case of skim milk and whole milk has received much less attention.

Buglia (10) demonstrated that homogenization increases the viscosity of whole milk, while it has practically no effect on skim milk. Wiegner (32) obtained results similar to those of Buglia for whole milk. Evenson and Ferris (14) also noted an increase in the viscosity of Pasteurized normal milk after it had been homogenized. Babcock (2) states that homogenization is very detrimental to the whipping qualities of cream, this effect being multiplied by raising the homogenization pressure. The work of Sherwood and Smallfield (28) is very interesting, since they studied, by means of a microscope as well as a viscometer, the effect of homogenization. They found that the rate of increase in viscosity of a sample of Pasteurized cream on aging was much greater if it had been homogenized, the increase being accompanied by a growing tendency of the globules to clump.

TABLE 12.—*Effect of homogenization on the viscosity of skim and whole milk*

Number of hours aged	Composition (percentage by weight) of—		Pressure (gms. per sq. cm.)	Viscosity (centipoise)	
	Fat	Total solids		Normal	Homogenized
6	0.11	8.87	277.3	1.383	1.362
			680.9	1.370	1.355
			980.4	1.360	1.351
8	.18	9.43	277.3	1.454	1.451
			680.4	1.432	1.422
			980.4	1.428	1.419
8	4.05	12.82	277.3	1.635	2.001
			680.1	1.623	1.998
			980.4	1.619	1.970
3	4.63	14.08	814.0	1.640	1.830
			814.0	1.640	1.831

Table 12 shows the effect of homogenization on the apparent viscosity of skim milk and whole milk. It is rather interesting to note that homogenization slightly decreases the viscosity of skim milk, while it materially increases the same physical property of whole milk. In the case of skim milk, it is probable that the state of subdivision of some of the protein clots which may be present is altered, when subjected to the violent physical forces of this treatment, so as to cause a slight diminution in viscosity.

A microscopical observation of the fat globules, before and after homogenization, showed that their average size had been greatly decreased. An increase in viscosity under the above conditions is quite in accord with the results of Odén (23), who studied the effect of decreasing the particle size on the viscosity. Hatschek (16) concluded that this augmentation was due to an adsorption film of the liquid phase around the particles, the thickness of which was independent of the particle size.

The same explanation can be used for the effect of homogenization on the viscosity of whole milk. Homogenization reduces the size of the fat globules, and at the same time increases the specific surface of the fat phase. Since the thickness of the adsorption film varies slightly if at all with the particle size, and the amount of adsorption depends upon the total surface, it can be seen that more of the skim-milk phase will be adsorbed, in the case of homogenized whole milk, and will no longer count as free liquid. This results in an increase in viscosity, due to a decrease in the volume of the dispersion medium and an increase in the volume of the dispersed phase. In other words, the augmentation of viscosity is probably due to the adsorption of the skim-milk phase by the fat globules, and consequently the homogenized whole milk becomes more viscous than ordinary milk of the same fat content. For example, Wiegner (32) reduced the average diameter of fat globules in milk from  $2.9 \mu$  to about  $0.27 \mu$  by the homogenization process. From viscosity measurements he calculated that in ordinary milk about 2 per cent of the casein is adsorbed by the fat, while the casein adsorbed in homogenized milk is about 25 per cent. This amount of protein adsorbed is probably too high due to his assumption that the adsorbed protein film had a density of about 1.4.

It was also found that when homogenized milk is aged at low temperatures for several days the viscosity rises. This is undoubtedly due to the altering of the proteins.

#### EFFECT OF AGING ON THE VISCOSITY OF SKIM MILK

When milk stands in contact with the atmosphere, at least a slight exchange of gases occurs. Adsorption of oxygen and nitrogen and a loss of dissolved carbon dioxide takes place. Probably there is a slow change in the solubility and equilibrium of the salts. Fat globules clump and rise, resulting in a cream layer at the surface. The proteins may be slowly denatured. The milk contains enzymes, some of which are inherent and other of bacterial origin. These enzymes alter the composition of the milk with aging. It is apparent that the study of the influence of aging on the physical properties of milk is important, since much of the market milk and cream is kept for some time at low temperatures before being used.

Kobler (20) believed that an initial increase in the viscosity of fresh whole milk occurred due to the gradual loss of air bubbles which were present, and that the later increase was due to the proteins, in which case the viscosity increased until the casein was precipitated. The loss of small air bubbles from the milk should, however, cause a decrease in viscosity as measured with a capillary viscometer. Burri and Nussbaumer (11) allowed a sample of whole milk to stand for 12 hours at about  $20^{\circ}\text{C}$ ., and found that the viscosity increased with aging. Evenson and Ferris (14) demonstrated that milk which had been aged for one week at  $3^{\circ}\text{C}$ . showed an increase in viscosity over that kept for one hour at  $30^{\circ}\text{C}$ . Dahlberg and Hening (13) also made an extensive study of the influence of aging on the viscosity of milk and cream and their results, in general, agree with those of the other workers.

Most of the recorded work on aging has been done with whole milk and cream. Much of the variation in the viscosity of whole milk, as has been previously shown, can be ascribed to a variation

in the degree of clumping of the fat globules. A study of the effect of aging on the viscosity of skim milk would show whether or not the augmentation noted on aging whole milk is due to a variation in the clumping of fat globules or to an actual increase in the viscosity of the skim milk phase.

TABLE 13.—*Effect of aging on the viscosity of skim milk*

Number of days aged	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
	Fat	Total solids		
0	0.19	9.18	983.9	1.500
			814.8	1.504
			682.2	1.506
			480.3	1.514
			278.1	1.522
			145.6	1.523
3	-----	-----	982.0	1.502
			814.8	1.504
			682.2	1.511
			480.3	1.517
			278.1	1.526
			147.0	1.531
5	-----	-----	68.0	1.530
			982.0	1.510
			814.8	1.513
			682.2	1.522
			480.9	1.528
			278.0	1.538
8	-----	-----	148.1	1.538
			74.0	1.546
			981.2	1.517
			814.8	1.525
			681.6	1.522
			483.0	1.541
			92.8	1.540

TABLE 14.—*Effect of aging on the viscosity of skim milk*

Number of days aged	Composition (percentage by weight) of—		Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
	Fat	Total solids		
0	0.12	9.87	981.2	1.500
			814.8	1.491
			681.6	1.506
			480.9	1.507
			278.1	1.519
			142.4	1.516
3	-----	-----	71.8	1.517
			982.3	1.518
			816.5	1.520
			683.2	1.525
			481.7	1.534
			278.8	1.539
5	-----	-----	139.2	1.547
			67.3	1.554
			981.7	1.518
			816.5	1.520
			683.2	1.525
			481.7	1.534
8	-----	-----	278.8	1.539
			139.2	1.547
			67.3	1.554
			983.7	1.557
			815.7	1.571
			683.3	1.568
			482.1	1.576
			278.8	1.586
			151.7	1.597

The samples of milk used in the foregoing experiments were obtained under the most sanitary conditions. Contamination was guarded against as much as possible. Sterilized bottles and pipettes were used throughout the work. The udder of the cow and the hands of the milker were thoroughly washed with dilute phenol and dried. The samples were milked directly into sterile cotton strainers which led into sterile Erlenmeyer flasks.

In the first set of experiments the effect of aging on the viscosity of skim milk was followed, and characteristic results are summarized in Tables 13 and 14 and in Figure 3.

Upon examining the data in Tables 13 and 14 and the curves in Figure 3, we note that, in general, the viscosity of skim milk increases with the progress of aging at low temperatures. Apparently this rise must be due to the changes that are taking place either in the com-

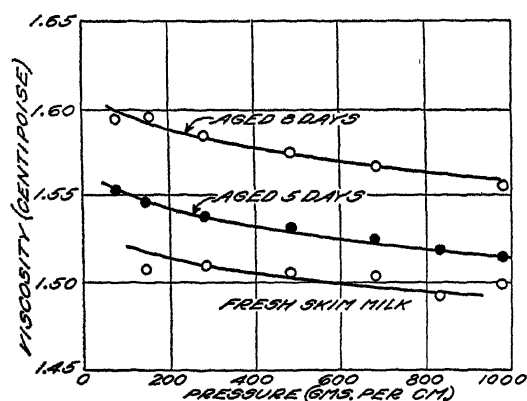


FIG. 3.—Increase in viscosity of skim milk with aging, and decrease in viscosity as shearing force is increased

position or the state of subdivision of the milk proteins, or both. These changes may be brought about by bacterial and milk enzymes, or they may be due partially to the constituents of the milk not being in equilibrium.

In order to gain some idea as to whether or not the changes which were taking place could be attributed to acidity, determinations of the  $P_H$  and titratable acidity were made during the

aging period. The results of this study are given in Table 15. In this series the milk was aged up to 25 days at about 3° C. and the effect of the storing on the viscosity,  $P_H$ , and titratable acidity were determined.

TABLE 15.—Effect of aging at 4°–6° C. for a long period of time on some of the physical properties of skim milk<sup>1</sup>

Number of days aged	Pressure (gm. per sq. cm.)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.	$P_H$	Titratable acidity (percentage lactic acid)
1	680.8	160.7	1.433	6.78	0.14
3	680.0	164.8	1.467	6.80	.13
6	680.8	170.6	1.521	6.81	.125
9	681.6	170.8	1.525	6.83	.12
15	680.8	174.0	1.551	6.84	.11
21	681.6	174.2	1.555	6.84	.11
25	(Unable to run because of the formation of finely divided coagulum.)				

<sup>1</sup> Total solids=8.98 per cent; fat=0.076 per cent.

It will be seen that the apparent viscosity of skim milk gradually increased from 1.433 to 1.555 centipoise after 21 days of aging. It

was impossible to determine the "viscosity" of the milk on the twenty-fifth day because very finely divided particles were evident throughout the milk. The  $P_H$  also increased very slightly with time, and the titratable acidity lessened.

#### THE EFFECT OF PASTEURIZATION ON THE VISCOSITY OF SKIM MILK

This series of experiments was devoted to the study of the effect of Pasteurization on the viscosity of skim milk. Most of the Pasteurization-viscosity studies had been carried out with whole milk and cream, and the results of various workers are not all concordant. Woll (33), Babcock and Russell (4, 5), Steiner (29), Evenson and Ferris (14), Dahlberg and Hening (13), and others have demonstrated that heating whole milk to Pasteurization temperatures causes a diminution of the viscosity. Achard and Stassano (1) and others do not agree with the majority of the workers on this subject, since they found a slight increase in the viscosity of milk when Pasteurized.

About the only work which has been done on the effect of Pasteurization on the viscosity of skim milk is that of Whitaker, Sherman, and Sharp (31). They found a slight drop in viscosity when skim milk is heated to Pasteurization temperatures.

In this investigation the raw skim milk was introduced into an Erlenmeyer flask and stoppered to prevent evaporation. A thermometer was inserted through the stopper, so that the temperature of the milk could be carefully watched. The flask was placed in a water bath and the temperature was raised to about 62° C. and maintained there for 30 minutes. During this entire time the flask was continuously agitated to prevent local heating. At the end of 30 minutes it was plunged into ice water and agitated until the milk was cooled to about 4° C.

The results are summarized in Table 16.

TABLE 16.—*Effect of Pasteurization on the viscosity of skim milk*<sup>1</sup>

Number of days aged	Raw milk		Pasteurized milk	
	Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.	Pressure (gm. per sq. cm.)	Viscosity (centipoise) at 25° C.
0	982.2	1.413	982.3	1.404
	682.4	1.423	683.2	1.410
	278.1	1.432	278.7	1.430
	117.9	1.439	118.0	1.429
	982.2	1.436	981.2	1.420
4	682.4	1.441	682.4	1.439
	277.1	1.452	278.1	1.446
	118.96	1.461	118.14	1.450

<sup>1</sup> Total solids=9.10 per cent; fat=0.15 per cent.

Table 16 brings out two important points; first, that Pasteurization slightly decreases the viscosity of skim milk. This agrees with the results of Whitaker, Sherman, and Sharp (31). Second, the average increase of the viscosity of Pasteurized milk due to aging, as found in these experiments, is almost the same as that of the raw milk. The process of Pasteurization should greatly reduce the number of organisms. In addition the organisms show very little action at a tempera-

ture of 3° to 4°C. These facts would indicate that the augmentation noted, which is practically the same in the Pasteurized and non-Pasteurized milk, is largely due to the altering of the proteins either by slow denaturation, by a shift of the equilibrium, by enzyme action, or by a combination of these factors.

#### THE EFFECT OF FREEZING ON THE VISCOSITY OF SKIM MILK

In the previous study it was noted that the viscosity of skim milk was slightly increased by aging at low temperatures. The next point considered was whether or not the viscosity of skim milk was affected by repeated freezing and thawing, and by aging in the frozen state.

The samples of milk used in this series of experiments were prepared in the same manner as those in the previous parts. Each sample was divided into several portions which were poured into sterile stoppered bottles. In every case one bottle of the milk was kept in ice water until the initial run was made, while the others were taken into a cold storage room where a temperature of -14° C. was maintained. Before making a viscosity determination the frozen sample was thawed out by immersing the bottle in lukewarm water. All samples were filtered before they were introduced into the viscometer. Filtration was necessary because of the presence of a small amount of finely divided precipitate in the frozen samples which would plug the viscometer.

The effect of repeatedly freezing and thawing, and of aging in the frozen state, on the viscosity of skim milk is summarized in Tables 17 and 18.

TABLE 17.—*Influence of aging on the viscosity of skim milk in the frozen state*<sup>1</sup>

Sample	Pressure (gm. per sq. cm.)	Number of days aged	Viscosity (centi- poise) at 25° C.
Raw sample 1.....	980.6	None.	1.505
	680.2	None.	1.513
	980.6	1	1.412
	680.2	1	1.420
	980.6	33	1.472
	680.2	33	1.475
Raw sample 2.....	980.6	None.	1.475
	680.9	None.	1.479
	980.6	31	1.389
	680.9	31	1.391
	979.9	None.	1.462
	679.5	None.	1.468
Pasteurized sample.....	979.9	1	1.446
	679.5	1	1.447
	979.9	20	1.461
	679.5	20	1.470

<sup>1</sup> Raw sample 1, total solids=9.63 per cent, fat=0.17 per cent; raw sample 2, total solids=9.42 per cent, fat=0.08 per cent; Pasteurized sample, total solids=9.06 per cent, fat=0.096 per cent; aged at -14° C.

In spite of the fact that all of the samples of raw skim milk were subjected to almost the same treatment before and after freezing, and were aged under the same conditions, the results do not appear to be as consistent as would be desirable. A slight precipitate was always noted in the frozen samples. It was necessary to filter the frozen milk before making the viscosity determination in order to remove this precipitate so that it would not plug the capillary. Since one freezing caused the formation of a small amount of precipitate and a

decrease in the viscosity as shown in Table 17 it seemed reasonable to suppose that repeated freezing and thawing would produce increasing amounts of precipitate. This apparently was not the case, however, as shown in Table 18. Part of the milk was repeatedly frozen and thawed once each day for a week. At the end of a week this sample had about the same viscosity as another aliquot which was kept frozen the entire time. In all of the cases where a slight viscosity drop was noted, it always occurred when the skim milk was frozen the first time, and a small amount of finely divided coagulum was formed. It is interesting to note that the viscosity of the frozen skim milk dropped with the first freezing, but when aged for some time in the frozen state at  $-14^{\circ}\text{C}.$ , it increased until the normal value of the milk before freezing was almost reached.

TABLE 18.—*Influence of repeated freezing and thawing on skim milk*<sup>1</sup>

Pressure (gm. per sq. cm.)	Number of days aged	Number of times frozen	Viscosity (centi- poise) at $25^{\circ}\text{C}.$
980.6	None.	None.	1.493
812.6			1.496
277.5			1.507
980.6	1	1	1.397
812.6			1.400
277.5			1.413
980.6	7	1	1.515
812.6			1.518
277.5			1.514
980.6	7	7	1.515
812.6			1.515
277.5			1.513

<sup>1</sup> Total solids=9.65 per cent; fat=0.10 per cent; aged at  $-14^{\circ}\text{C}.$

## EFFECT OF DILUTION ON THE VISCOSITY OF SKIM MILK

Many workers in searching for a shorter and a more convenient method for the determination of total solids and also for the detection of possible cases of watering, have attempted to establish a straight line relationship between the viscosity of whole milk and its composition. In the previous parts of this paper, the way in which the viscosity can vary depending on the previous treatment of the milk has been noted. This variation is especially marked in the case of the effect of the fat on the viscosity. The general application of the various formulas proposed assumes a constancy of the effect of the various constituents of milk on its viscosity. Such an assumption is unwarranted except to a very limited extent. It should be possible to eliminate one of the variable factors by the removal of the fat, but even under such conditions any relationship between the viscosity and the composition would apply only to milk samples which had the same previous treatment, and such a relationship would therefore not be general in its application.

Babcock (3) developed a very empirical formula which was supposed to express the relationship between the viscosity and the total solids. Babcock and Russell (4) later limited the use of this method by showing that the effect of fat was not constant but varied with the state of aggregation. Both Bogdan (9) and Oertel (24) concluded that the viscosity was closely related to the composition of the milk. However, the inner resistance to flow was especially sensitive to the fat

content. Taylor (30) believed that the total solids not fat could be calculated, if one knew the viscosity and the percentage of fat, by means of a formula which was based on the assumption that fat has a constant effect on the viscosity and varies only with the quantity. Babcock (3) showed that this relation did not hold, since the influence of a given amount of fat on the viscosity of milk is variable. Evenson and Ferris (14) compared Pasteurized normal milk with remade milk by means of the viscosity method. They assumed that a linear relation existed between the difference in the viscosity of milk and of water, and the total solids. Kooper (21) attempted to show that viscosity was a linear function of the total solids content, and could be used to detect cases where milk had been diluted with water. He believed that if one knew the viscosity of a diluted milk, the percentage of added water could be calculated by means of a constant (0.1384) which he obtained by dividing the average viscosity (1.588) by the average total solids content (11.472) of 50 samples of milk. This so-called constant is based on a rather illogical assumption, namely, that at zero concentration (water) the viscosity would also be zero. On the other hand, when he used this constant to calculate the added water, he subtracted the viscosity of water from the viscosity of the milk.

The effect of aging, Pasteurization, and the state of aggregation of the fat particles on the viscosity values has already been pointed out. Samples of milk might contain the same amount of milk solids not fat, and the composition of the milk solids not fat still shows enough variation to affect the viscosity.

In this set of experiments the viscosity of skim milk, systematically watered, was considered.

The skim milk was prepared as previously described, and was diluted with freshly distilled water. The water was always run into the milk from a burette until the desired dilution was made. The diluted samples were next stirred and left for two hours to allow the constituents to come to equilibrium.

The results of this study are summarized in Tables 19 and 20, and are graphically shown in Figure 4.

TABLE 19.—*Effect of dilution on the viscosity of fresh Guernsey skim milk from morning milking*<sup>1</sup>

Dilution water and milk (by volume)	Milk solids not fat (percentage by weight)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.
0 100	9.34	163.0	1.457
10 90	8.41	154.9	1.381
20 80	7.47	147.8	1.319
30 70	6.54	141.4	1.258
40 60	5.77	128.8	1.143
50 50	4.77	112.2	0.993
100 0	0.00	-----	0.894

<sup>1</sup> Total solids=9.46 per cent; fat=0.12 per cent; aged 6-8 hours at 4° C.; pressure=681.9 grams per square centimeter.



TABLE 20.—*Effect of dilution on the viscosity of fresh Guernsey skim milk from morning milking*<sup>1</sup>

Dilution water and milk (by volume)	Milk solids not fat (percentage by weight)	Time of flow (seconds)	Viscosity (centipoise) at 25° C.
0 100	9.27	161.8	1.440
10 90	8.34	153.4	1.365
20 80	7.42	146.8	1.305
30 70	6.49	140.1	1.245
50 50	4.63	127.4	1.130
80 20	1.85	110.8	0.980
100 0	0.00	-----	0.894

<sup>1</sup> Total solids=9.40 per cent; fat=0.13 per cent; aged 6-8 hours at 4° C.; pressure=680.2 grams per square centimeter.

The data plotted in Figure 4 indicate that the relation between the viscosity and the milk solids not fat is expressed by a line slightly curved. However, for practical purposes where slight dilutions are being considered, a straight line relationship between the apparent viscosity of skim milk and its composition can be assumed.

A 10 per cent dilution resulted in lowering the viscosity of skim milk from 1.457 to 1.381 centipoise in one case, and from 1.440 to 1.365 in another. This means that the former was decreased by 0.076 centipoise and the latter by 0.075 centipoise by the same dilution. In a previous part a rise in viscosity of skim milk almost as great as the decrease noted above was observed, by simply allowing the milk to remain at a low temperature for several days. Since fresh skim-milk samples have occasionally been found to have a viscosity from 1.324 to 1.592 the viscosity method alone would not be reliable for detecting small amounts of added water.

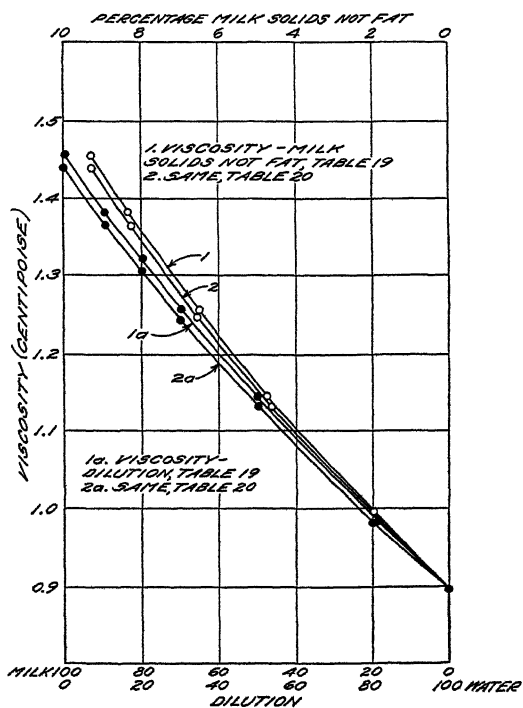


FIG. 4.—Decrease in viscosity of skim milk with progressive dilution with distilled water compared with the percentage of skim milk in the mixture and with the total solids content

## DISCUSSION

The experiments reported here have shown that even the viscosity coefficient of skim milk is dependent on the shearing force used in determining it. The percentage variation in the viscosity coefficient

of normal skim milk through the range of shearing force used was small, and this slight variation could therefore be disregarded for many purposes; but the fact that such a variation does exist should not be lost sight of. The viscosity of milk can not be accurately expressed as a single value without at the same time giving the shearing force under which it was obtained.

In order to express the pressure in terms of the shearing force which is independent of the size and length of the capillary, the following relationship between shearing force and pressure was used.

$$F = \frac{rgP}{2l} \quad (8)$$

where  $F$  is the shearing force, and  $r$  and  $l$  are the radius and length of the capillary,  $P$  the pressure in grams per square centimeter, and  $g$  the gravitational constant.

The radius (0.0108 cm.) of the capillary was obtained approximately by substituting the value for  $C$  in equation (3).

Substituting the values in equation (8), the following relation between shearing force and pressure is given:

$$F = 0.530 P \quad (9)$$

The shearing force used in this work as calculated by equation (9) varied from about 36 to 780 dynes. The curves (see fig. 2) bent more sharply at the lower shearing pressures, while at the higher pressures the viscosity coefficient approached more nearly a constant limiting value.

The viscometers which are frequently used for determining the viscosity of milk, such as the ordinary Ostwald viscometer, probably operate at shearing forces between 25 and 100 dynes. They thus operate on that part of the curve where the viscosity coefficient is changing most rapidly with shearing force. Viscometers of this type must be constructed very nearly alike in order that they may give the same viscosity coefficient with the same sample of milk.

The decrease in the viscosity coefficient with increasing shearing force is not due to the breaking down of a structure in the skim milk; otherwise repeatedly running the same sample through the capillary would accomplish the same purpose. Also the increase in the viscosity of skim milk by aging can hardly be explained on the basis of a structure formed, for if this were the case one would expect that the structure would be broken down again by repeatedly running the aged sample through the capillary. The experiment with colostrum skim milk showed that the viscosity actually increased while the milk was being run repeatedly through the capillary.

Homogenization of milk and cream shows important viscosity effects; the fat globules are greatly reduced in size in both; the fat globules in the homogenized cream are markedly clumped, but clumps are practically absent in homogenized milk. This is the reason why homogenized milk does not cream to any appreciable extent, and explains why the viscosity values are so uniform with homogenized whole milk as compared with raw whole milk.

Repeatedly running normal skim milk and homogenized whole milk through the capillary of the viscometer at shearing forces from 360 to 550 dynes had no effect on the viscosity coefficient. Raw

whole milk subjected to the same treatment decreased in viscosity during the first 10 times through the capillary and after that up to 25 times through the capillary practically no further decrease in viscosity occurred. Microscopic observations indicated that this decrease in viscosity was due to the breaking up of the clumps of fat globules. Experiments in another connection have shown that as the temperature is raised the fat globules are held in the clumps less tenaciously so that it is highly probable that the decrease in viscosity due to running raw whole milk repeatedly through the capillary would have been much greater if the experiment had been carried out at a lower temperature. These experiments indicate that mechanical agitation in which air is not incorporated in the milk would have no effect on the viscosity of skim milk or homogenized whole milk but would tend to decrease the viscosity of milk in which clumps of fat globules are present.

The experiments on the effect of freezing on the viscosity were not very extensive, but they probably give an indication of the general influence of freezing on skim milk.

The decrease in the viscosity of skim milk due to dilution with water was shown to be represented by a slightly curved line. Attention should be called to the fact that as milk is diluted with water the hydrogen ion concentration decreases, as shown by Sharp and McInerney (27). This change in hydrogen ion concentration might produce a change in the hydration of the proteins so that until further experiments are carried out one is not justified in using this data in studying the relation between viscosity and concentration under the same conditions.

There is little doubt that taking a large number of samples, a relation exists between the total solids and the viscosity of milk, but because of the large probable error and the many factors which effect it, such as aging, Pasteurization, clumping of the fat globules, variation in the composition of the total solids, etc., the use of the viscosity as a substitute for the total solids determination of individual samples of milk is of limited value. Because of these same variables and due to the possibility of a wide variation in the viscosity of normal milk, the viscosity determination alone can not be used as a test for small amounts of added water.

### CONCLUSIONS

The viscosity coefficient of whole milk, condensed skim milk, and even skim milk is not independent of the rate of shear, but decreases as the rate of shear is increased, approaching a constant value at high rates of shear.

The bending of the viscosity pressure curve is greatest in the region of shearing force which is commonly used for determining the viscosity of milk and milk products; therefore different investigators determining the viscosity of the same sample of milk would hardly obtain exactly the same viscosity coefficient, unless the viscometers which they used were nearly identical.

Mechanical agitation may cause a decrease in the viscosity of milk containing clumps of fat globules due to the breaking up of these clumps. Mechanical agitation produces no decrease in the viscosity of skim milk or of homogenized milk.

Skim milk progressively increased in viscosity with age. The aged skim milk could not be brought back to the viscosity value which it had when fresh by repeatedly running the aged sample through the capillary.

Homogenization caused a distinct rise in the viscosity of whole milk, while it caused practically no change in the viscosity of skim milk.

Pasteurization of skim milk at 62° C. for 30 minutes caused a slight decrease in viscosity when determined at 25° C.

Frozen skim milk held one day showed a decreased viscosity, but after it had aged in the frozen state for several days the viscosity increased to near that for the fresh sample.

Repeatedly freezing and thawing skim milk once each day for seven days produced the same effect on the viscosity as holding aliquot for the same length of time in the frozen condition.

The viscosity values obtained after diluting skim milk with various amounts of water fell on a slight curve, showing that the viscosity is not strictly a linear function of the total solids.

These experiments indicate that the viscosity of a sample of milk does not necessarily indicate accurately the total solids content except possibly for very restricted groups of samples.

#### LITERATURE CITED

- (1) ACHARD, G., and STASSANO, H.  
1925. QUELQUES CONSTANTES PHYSICO-CHIMIQUES DU LAIT CRU ET DU LAIT PASTEURISÉ SELON DIFFÉRENTS PROCÉDÉS. *Compt. Rend. Soc. Biol. [Paris]* 93: 708-710.
- (2) BABCOCK, C. J.  
1922. THE WHIPPING QUALITY OF CREAM. U. S. Dept. Agr. Bul. 1075, 22 p., illus.
- (3) BABCOCK, S. M.  
1887. REPORT OF THE CHEMIST. N. Y. State Agr. Expt. Sta. Ann. Rpt. (1886) 5: 297-334, illus.
- (4) ——— and RUSSELL, H. L.  
1896. ON THE RESTORATION OF THE CONSISTENCY OF PASTEURIZED MILK AND CREAM. *Wis. Agr. Expt. Sta. Ann. Rpt.* 13: 81-94, illus.
- (5) ——— and RUSSELL, H. L.  
1896. CONDITIONS AFFECTING THE CONSISTENCY OF MILK. *Wis. Agr. Expt. Sta. Ann. Rpt.* 13: 73-80, illus.
- (6) BINGHAM, E. C.  
1922. FLUIDITY AND PLASTICITY. 440 p., illus. New York and London, McGraw-Hill Book Co., Inc.
- (7) ——— and JACKSON, R. F.  
1918. STANDARD SUBSTANCES FOR THE CALIBRATION OF VISCOMETERS. U. S. Dept. Com., Bur. Standards Sci. Paper 298, p. 59-86, illus.
- (8) ——— and WHITE, G. F.  
1912. FLUIDITÄT UND DIE HYDRATTHEORIE. I. DIE VISKOSITÄT VON WASSER. *Ztschr. Phys. Chem.* 80: 670-686, illus.
- (9) BOGDAN, S.  
1905. SUR L'EMPLOI DE LA MÉTHODE VISCOSIMÉTRIQUE POUR LE CONTRÔLE DU LAIT. *Ann. Chim. Analyt.* 10: 90-92.
- (10) BUGLIA, G.  
1908. UEBER EINIGE PHYSIKALISCH-CHEMISCHE MERKMALE DER HOMOGENISIERTEN MILCH. *Ztschr. Chem. u. Indus. Kolloide* 2: [353]-354.
- (11) BURRI, R., and NUSSBAUMER, T.  
1909. ÜBER OBERFLÄCHENSpannungs-UND VISCOSITÄTSBESTIMMUNGEN BEI KUHMITCH UNTER VERWENDUNG DES TRAUBESCHEN STALAGMOMETERS. *Biochem. Ztschr.* 22: [90]-102.
- (12) CAVAZZANI, E.  
1905. VISKOSITÄT DER MILCH. *Zentbl. Physiol.* (1904) 18: [841]-845.

- (13) DAHLBERG, A. C., and HENING, J. C.  
1925. VISCOSITY, SURFACE TENSION, AND WHIPPING PROPERTIES OF MILK AND CREAM. N. Y. State Agr. Expt. Sta. Tech. Bul. 113, 42 p., illus.
- (14) EVENSON, O. L., and FERRIS, L. W.  
1924. THE VISCOSITY OF NATURAL AND REMADE MILK. Jour. Dairy Sci. 7: 174-188, illus.
- (15) GALDI, F.  
1909. INFLUENZA DELLA PRESSIONE E DEL PESO SPECIFICO SUL COEFFICIENTE VISCOSIMETRICO RELATIVO DEI LIQUIDI ORGANICI IN RAPPORTO ALLE SOLUZIONI CRISTALLOIDI E COLLOIDALI. Gior. Internaz. Sci. Med. [n. s.] 31: [157]-176, [205]-225.
- (16) HATSCHKE, E.  
1912. DIE EXISTENZ UND WAHRSCHEINLICHE DICKE VON ADSORPTIONS-HÜLLEN AUF SUSPENSION-TEILCHEN. Ztschr. Chem. u. Indus. Kolloide 11: 280-284.
- (17) ———  
1927. THE APPARENT VISCOSITY OF COLLOIDAL SOLUTIONS. Jour. Phys. Chem. 31: [383]-392, illus.
- (18) ———, and JANE, R. S.  
1926. DIE VISKOSITÄT VON SUSPENSIONEN STARRER TEILCHEN UND IHRE ABHÄNGIGKEIT VOM SCHERGEFÄLLE. Kolloid. Ztschr. 40: 53-58, illus.
- (19) HESS, W. R.  
1920. BEITRAG ZUR THEORIE DER VISKOSITÄT HETEROGENER SYSTEME. Kolloid Ztschr. 27: 1-11, illus.
- (20) KOBLER, B.  
1908. UNTERSUCHUNGEN ÜBER VISKOSITÄT UND OBERFLÄCHENSpannung DER MILCH. Arch. Gesamt. Physiol. 125: 1-72, illus.
- (21) KOOPER, W. D.  
1914. DIE BESTIMMUNG DER VISKOSITÄT DER MILCH ALS MITTEL ZWECKS FESTSTELLUNG EINES STATTGEHABTEN WASSERZUSATZES. Milchw. Zentbl. 43: [169]-179, [201]-208, illus.
- (22) MOJONNIER, T., and TROY, H. C.  
1925. THE TECHNICAL CONTROL OF DAIRY PRODUCTS. Ed. 2, 936 p., illus. Chicago, Illinois, Mojonnier Bros. Co.
- (23) ODÉN, S.  
1912. PHYSIKALISCH-CHEMISCHE EIGENSCHAFTEN DER SCHWEFELHYDROSOLE. Ztschr. Physik. Chem. 80: 709-736, illus.
- (24) OERTEL, E.  
1908. UEBER DIE VISKOSITÄT DER MILCH. (Diss. Leipzig.) [Original not seen. Abstract in Milchw. Zentbl. 7: 137. 1911.]
- (25) ROTHLIN, E.  
1919. ÜBER DIE METHODIK DER VISCOSITÄTSBESTIMMUNG BEI ORGANISCHEN KOLLOIDEN. Biochem. Ztschr. 98: [34]-91, illus.
- (26) ———  
1920. ÜBER DIE VISCOSITÄTSBESTIMMUNG BEI ORGANISCHEN KOLLOIDALEN LÖSUNGEN. Verhandl. Schweiz. Naturf. Gesell. 100 (2): 141-143. (Atti. Soc. Elvetica Sci. Nat.)
- (27) SHARP, P. F., and McINERNEY, T. J.  
1926. THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF MILK, WHEY, AND CREAM. Jour. Biol. Chem. 70: 729-758, illus.
- (28) SHERWOOD, F. F., and SMALLFIELD, H. L.  
1926. FACTORS INFLUENCING THE VISCOSITY OF CREAM AND ICE CREAM. Jour. Dairy Sci. 9: 68-77.
- (29) STEINER, R.  
1901. BEITRÄGE ZUR KENNNTNIS DES EINFLUSSES DER PASTEURISIERUNG AUF DIE BESCHAFFENHEIT DER MILCH UND AUF DEN BUTTERUNGS-PROZESS. Milchw. Ztg. 30: [401]-403.
- (30) TAYLOR, H. B.  
1913. SOME PHYSICO-CHEMICAL MEASUREMENTS ON MILK. Roy. Soc. N. S. Wales, Jour. and Proc. 47: 174-192, illus.
- (31) WHITAKER, R., SHERMAN, J. M., and SHARP, P. F.  
1927. EFFECT OF TEMPERATURE ON THE VISCOSITY OF SKIMMILK. Jour. Dairy Sci. 10: [361]-371, illus.

- (32) WIEGNER, G.  
1914. UEBER DIE AENDERUNG EINIGER PHYSIKALISCHER EIGENSCHAFTEN  
DER KUHMITCH MIT DER ZERTEILUNG IHRER DISPENSEN PHASEN.  
Kolloid Ztschr. 15: [105]-123, illus.
- (33) WOLL, F. W.  
1896. THE EFFECT OF PASTEURIZATION AND STERILIZATION ON THE VIS-  
COSITY AND FAT GLOBULES OF MILK AND CREAM. Wis. Agr.  
Expt. Sta. Ann. Rpt. (1895) 12: 164-173.



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## PATHOGENICITY OF TWO STRAINS OF THE TOMATO-WILT FUNGUS, *FUSARIUM LYCOPERSICI* SACC.<sup>1</sup>

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### INTRODUCTION

In the studies of tomato wilt (*Fusarium lycopersici* Sacc.) conducted to date, major stress has been laid upon the development of wilt-resistant varieties of tomatoes (15, 16, 17, 24, 33)<sup>3</sup> and upon the relation of the causal organism and disease production to different environmental factors (20, 21, 25, 27). Reports that tomato varieties of established resistance in the areas where they were developed were succumbing to wilt in other sections of the country (22, 26) have indicated that the possible existence of different species, varieties, or forms of the wilt pathogene should be investigated. White (34) in studying this phase of the wilt problem made various tests of 24 strains<sup>4</sup> of *F. lycopersici* received from different sections of the country. By means of physiologic tests, he found that the various strains could be placed in one of two large groups. Members of the two groups differed markedly in virulence; however, White's tests were not made under conditions ideal for infection, and so it was deemed advisable to test the pathogenicity of a typical member of each group, on several varieties of tomato, under controlled environmental conditions. The present paper, and one dealing with the relation of excretory products of *F. lycopersici* to the production of wilt (18) are complementary to the investigations conducted by White (34).

The organism hereafter mentioned as strain A was isolated by R. P. White in 1922 at Manhattan, Kans., and designated by him strain 68. The other organism, strain B, was isolated in Louisiana by C. W. Edgerton and was reisolated at Manhattan in 1923. This culture, numbered strain 127 by White, was originally from Edgerton's culture, Louisiana 397. Both cultures were pure lined by White.

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<sup>2</sup> The writer wishes to express his gratitude to Dr. L. R. Jones and Dr. J. G. Dickson, of the University of Wisconsin, under whose immediate supervision the studies herein reported were conducted, for their inspirational and practical guidance. Thanks are also due Dr. R. P. White, formerly of this institution and now connected with the staff of the New Jersey Agricultural Experiment Station, and Dr. J. C. Walker, of the University of Wisconsin, for advice and critical reading of the manuscript, and to Mrs. H. H. Haymaker for assistance in much of the routine work of obtaining and tabulating data and in preparation of the results for publication.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 693.

<sup>4</sup> The word "strain" as used in this paper is in no way synonymous with variety, specialized race, or other terms used to designate specialization within a species. It simply refers to isolations of the fungus obtained from different localities.

CHARACTERISTICS OF THE TWO STRAINS IN CULTURE<sup>5</sup>

The relatively few references (16, 34) to cultural characteristics of *Fusarium lycopersici* indicate that strains isolated from different sections of the country vary noticeably in such characters as color formation, spore production, nature of the mycelial growth, zonation, and others. To determine the relationship between strains A and B, comparative cultural characteristics were observed on 14 kinds of media. It was found that the two strains, while exhibiting many characteristics in common, varied distinctly as to (1) color production, (2) sporulation, and (3) nature and elevation of mycelium.

Strain A, almost without exception, produced much more color, varying from salmon pink on Richards' solution to deep, vinaceous red in plate cultures of acidified potato agar at temperatures ranging from 20° to 32° C. The mycelium of strain B varied from white to pale pink, the former predominating.

Strain A, without exception, sporulated much more profusely than strain B. Microconidia of strain A developed in abundance in practically all kinds of media, a characteristic which was not shared by strain B. Macroconidia were produced sparingly by strain A on several kinds of media; with strain B they were found but once. Varying the temperature and P<sub>H</sub> of the media had no effect in changing this relationship between the two strains.

Strain A could be distinguished without difficulty because of its luxuriant development of aerial mycelium. The hyphae of strain B, on the contrary, were usually submerged, though occasionally powdery areas developed in Petri-dish cultures owing to a light growth of surface mycelium.

While the strain characteristics observed by White (34) and those here recorded agreed as to color production and nature of the mycelium, they differed markedly as to sporulation. White found that strain B produced both macro and micro conidia abundantly when grown on acidified potato agar, while strain A was practically sterile when grown under similar conditions.

Such a marked variation in results is difficult of explanation. Strain B has been shown to be extremely variable, as reisolations from diseased tomato seedlings differed from the parent strain in many ways, though they all retained the characteristic of approximate sterility as to spore production. The change in the nature of the fungus may have been due to deterioration through prolonged growth in culture (3), but the fact that strain B had been cultured a year less than strain A would indicate that the probable cause of the change was the accidental transfer of saltation material not evident in test-tube cultures. Brown (6) has shown that certain *Fusaria* saltate freely. He has concluded that fungi do not deteriorate gradually in culture, apparent changes of this kind being due to the accidental transfer of mycelium from a saltation sector. Edgerton and Moreland (15, 16) have demonstrated also that certain strains of *Fusarium lycopersici* deteriorate only slightly when grown in culture during an extended period of time.

<sup>5</sup> Detailed observations may be obtained from the following: HAYMAKER, H. H., TOMATO WILT: PATHOGENICITY OF TWO STRAINS OF *FUSARIUM LYCOPERSICI* SACC. AND THE RELATIVE TOXICITY OF THEIR EXCRETORY PRODUCTS, 1927. [Unpublished thesis. Copy on file, Univ. Wis., Madison.]



## OCCURRENCE OF SALTATION STRAINS

In an effort to obtain more rapid sporulation, strain B was exposed to a variety of conditions. When grown at 24° C. on potato-dextrose agar acidified with one drop of 25 per cent lactic acid per tube, strain B produced marginal saltation<sup>6</sup> sectors which differed from the remainder of the colony in color and in the nature of the mycelium. These sectors were apparently similar in nature to those occurring in other genera of fungi described by Christensen (9, 10) and Leonian (20, 21).

Transfers from these areas remained true to the type of the parent sectors. Petri-dish cultures, obtained from single spores, were vinaceous red in color, and there was an abundance of aerial mycelium which gave the culture a cottonlike appearance. The aerial mycelium in one isolation developed microconidia in abundance; in another, both micro and macro conidia were produced profusely. Of the several isolations made, two, designated as strains C and D, were kept in culture and later were tested for comparative pathogenicity. Throughout a series of transfers there has been no change in the characteristics of the two saltation strains.

This evidence, indicating anew the decided variability of strain B, provides additional weight for the assumption that this form was probably different from that of White's original strain 127, and that a new type had developed in the manner described by Brown (6).

RELATION OF TEMPERATURE TO GROWTH ON POTATO-DEXTROSE AGAR<sup>7</sup>

White (34) employed differences in growth rates on potato-dextrose agar as one of the means by which he separated 24 strains into two large groups. In view of the differences shown in cultural tests between his culture No. 127 and the writer's strain B, it was considered advisable to conduct a similar experiment to determine if strain B had changed in this respect also. Growth was determined by the increase in the size of the colonies on solid culture media, as it has been shown that this method is desirable in many respects (5). Standard methods used for tests of this kind were employed, and precautions were taken to make all environmental conditions as nearly uniform as possible. Two measurements were made of each colony and a large number of cultures was maintained at each temperature; so the final average was obtained from the results of either 8 or 16 determinations. The results are shown in Table 1 and are represented graphically by Figure 1. Similar results were obtained from two additional experiments in which both plain and acidified potato agar were used and in which the same methods were employed.

<sup>6</sup> The majority of investigators have spoken of similar sectors as mutation sectors. It has been suggested by others (3, 4, 6) that the term "mutation" should not be used to designate areas of this type in fungous cultures, inasmuch as the nature of their origin and the nuclear history involved is not understood. Consequently, in this paper, the more general term "saltation" as suggested by Stevens (29) will be used, thus including the possibilities that the sectors may have originated as a result of mutations, or of segregations following some type of nuclear fusion.

<sup>7</sup> The following formula was used: Potato, 200 gm.; dextrose, 20 gm.; agar-agar, 25 gm.; distilled water to make 1,000 c. c.

TABLE 1.—The average diameter (in mm.) of colonies of strains A and B on potato-dextrose agar, showing the extent of radial expansion during the last five days of a seven-day period <sup>a</sup>

Strain	Average diameter of colonies (mm.) at—									
	6° C.	8° C.	11° C.	15° C.	20° C.	24° C.	28° C.	31° C.	33° C.	38° C.
A -----	0	1	17	31	42	61	63	46	22	0
B -----	1	1	18	34	42	61	64	57	36	0

<sup>a</sup> 8 colonies were measured at each of the more critical temperatures (24°, 28°, 31°) and 4 colonies at the remaining temperatures.

It is evident that there is little difference between the growth rates of the two strains if growth be expressed by radial expansion of the

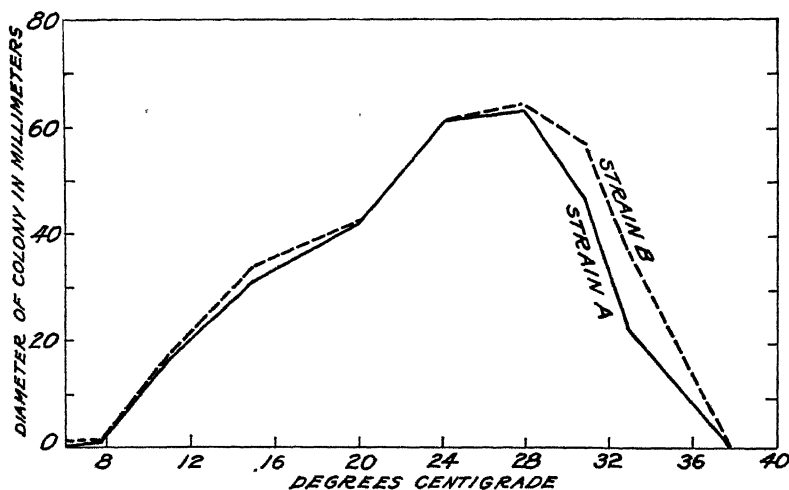


FIG. 1.—Temperature-growth curves of strains A and B of *Fusarium lycopersici* grown on potato-dextrose agar

colony on the medium used. The most marked difference is shown at temperatures above 28° C., at which strain B is markedly the more vigorous. This is in accord with White's findings.

#### COMPARATIVE PATHOGENICITY OF THE TWO STRAINS

Differences in virulence of strains of *Fusarium lycopersici* have been noted by various investigators (11, 15, 16). Both Clayton and Edgerton were of the opinion, however, that this variation was of no great significance. White (34) in testing the relative pathogenicity of 24 isolations from different parts of the United States, found decided differences in strain virulence and concluded from this evidence and other physiologic tests that the various strains could be grouped into two large subdivisions, which possibly merited distinction as varieties within the species. The possibility that *Fusarium*

*lycopersici* is composed of distinct races is suggested by other observations.<sup>8</sup>

The present experiment was designed to test the virulence, under carefully controlled environmental conditions, of a typical representative of each of the two major groups proposed by White. Seedlings were used, as it has been demonstrated that infection studies in which tomato seedlings are treated in the greenhouse produce results comparable to those obtained in field experiments with more mature plants (15). Seed of the standard commercial varieties was obtained from seed houses, while that of the resistant varieties was kindly furnished by F. J. Pritchard, C. W. Edgerton, and R. P. White.

#### METHODS

The methods involved in the preparation of the soil and the inoculum and the treatment of the host plants were essentially the same for all of the pathogenicity experiments. A soil composed of 1 part sand, 1 part manure, and 3 parts of field soil was used for all of the trials. After the soil was sterilized sufficient distilled water was added to raise the moisture to 70 per cent of its water-holding capacity, the amount required ranging from 25 to 27 per cent of the weight of air-dry soil.

Seed of different varieties of tomato was planted in 6-inch cans, which were then placed in soil temperature tanks. Soil and air temperatures of approximately 28° C. were maintained and the soil moisture was kept at a point favorable for infection (11, 12). When the plants were in the second-leaf stage they were thinned, and the soil was inoculated by pouring on a heavy spore and mycelial fragment suspension obtained either from Petri-dish cultures grown on potato-dextrose agar, or from flask cultures grown on steamed barley kernels. A standardized method of producing the inoculum was employed, so that each can of plants was treated with 100 c. c. of a composite suspension obtained from the same number of 10-day-old Petri-dish cultures as there were cans to be inoculated.

The treated plants were examined each day for signs of wilting. Those that showed definite symptoms of the disease were removed, sectioned to disclose the blackened vascular system, and in case of doubt the fungus was isolated from affected tissues. At the conclusion of each experiment, usually 30 days after inoculation, the stems of the plants were sectioned at the soil line, and were examined for bundle blackening. The fungus was reisolated without difficulty from affected plants by placing a small portion of a blackened bundle on potato agar. At the conclusion of each experiment the identity of the organism was obtained after isolations were made from representative diseased plants from each can.

#### RELATION OF SPORE LOAD TO INFECTION

In the first experiments conducted in which the pathogenicity of the two strains was compared, strain A proved to be much the more virulent. It was recognized, however, that the relative pathogenicity

<sup>8</sup> F. P. McWhorter of the Virginia Truck Experiment Station, in a letter to L. R. Jones, stated that the ordinary varieties of wilt-resistant tomatoes were very unsatisfactory when planted in certain sections of Virginia where wilt was severe. He suggested that there existed distinct specialized strains or races of *Fusarium lycopersici*. His investigational work indicated that he might be able to prescribe certain varieties of tomato for use in different localities. He thus intimated that different strains of the pathogene and varieties of the host exhibited a reciprocal relationship in respect to pathogenicity and susceptibility.

could not be determined until additional information was available with regard to the quantity of inoculum necessary to produce infections characteristic of each strain.

In the earlier trials, the inoculum for each can of 25 seedlings consisted of the conidia obtained from a single Petri-dish culture, suspended in 100 c. c. of water. It was soon realized, however, that this inoculum might not be fairly representative of strain B, which sporulated much less profusely than strain A. A spore count, made with a Levy counting chamber, of the two inocula revealed the fact that microconidia from strain A were being applied at the approximate rate of 10,000,000 per cubic centimeter, while those of strain B numbered only 687,000 per cubic centimeter, a disparity in the ratio of about 15 to 1. Observational evidence indicated, however, that this difference might not be of importance, as conidia of strain B were found to have a higher percentage of viability than those of strain A. When an inoculum prepared in this manner was used to inoculate cultures in Richards' solution, the colonies from

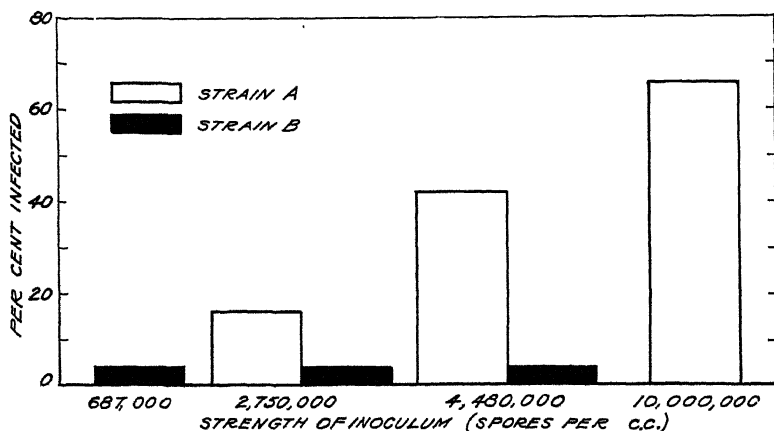


FIG. 2.—Relation of spore load to infection by strains A and B; plants grown in artificially inoculated soil at temperatures ranging from 26° to 28° C.

strain A seldom outnumbered those of strain B by more than two to one. The relative vegetative vigor of the two strains in Richards' solution was not determined by the dry-weight method, but observational evidence indicated that strain B was the more vigorous of the two.

In order to determine the importance of spore load, two experiments were conducted to test the relative virulence of the two strains; (1) when the inocula were obtained from similar amounts of Petri-dish material, and (2) when the number of microconidia was equalized. In the first experiment, seedlings of two varieties of tomatoes, Chalk's Early Jewel and New Stone planted October 28, were inoculated with representative samples of suspensions of spores of the two strains, the spores having been previously counted and the number adjusted until the inoculum of each strain contained approximately 2,750,000 microconidia per cubic centimeter. The plants as usual were inoculated while in the second-leaf stage, November 17, the final records being taken 30 days later, December 17. The plants

were grown at a soil temperature of 26° to 28° C. and an air temperature ranging from 25° to 32°.

This experiment was repeated with two other varieties, Norton and a Kansas selection, 9A. The inoculum was of the same type, but was obtained from flask cultures of the two strains growing on steamed barley kernels. The spore load was equalized as before, but the number of conidia was increased, so that the concentration was nearly twice that of the previous experiment. The plants were grown under similar conditions and the data were obtained as before. The experiment was conducted between December 6 and February 3. The results of both series are shown in Table 2 and are represented graphically by Figure 2.

TABLE 2.—*Effect of spore load on infection of tomatoes of different varieties by strains A and B of Fusarium lycopersici*

Strain of <i>F. lycopersici</i>	Tomato variety	Percentage of plants infected by inoculum of different strengths *			
		687,000 spores per cubic centimeter	2,750,000 spores per cubic centimeter	4,480,000 spores per cubic centimeter	10,000,000 spores per cubic centimeter
A	Chalk's Early Jewel.....		22		77
	New Stone.....		10		53
	Kansas selection 9A.....			56	58
	Norton.....			28	61
	Average.....		16	42	65
B	Chalk's Early Jewel.....	6	6		
	New Stone.....	3	3		
	Kansas selection 9A.....	4		3	
	Norton.....	3		5	
	Average.....	4	4.5	4	

\* In each trial the plants varied in number between 100 and 230.

The results of these two experiments show that the infecting power of strain A is diminished by reducing the number of conidia from that used in the preliminary experiments, while that of strain B is not affected by increasing the spore load. Hence it is evident that the relative pathogenicity of the two strains was tested more accurately by applying the inoculum from a single Petri-dish culture to each can, as this treatment provided more nearly optimal opportunity for infection by each strain.

#### RELATION OF TYPE OF INOCULUM TO PATHOGENICITY

White (34), in comparing the virulence of strains A and B, found the latter to be decidedly the more pathogenic of the two. This was in direct contrast to the results obtained in early experiments by the writer, in which A proved to be more virulent in every case. It was thought that the contradiction in results might have been due to the type of inoculum used, as White had inoculated the soil with a heavy concentration both of spores and mycelium obtained from flask cultures on steamed wheat kernels, while in experiments conducted by the writer, the soil was inoculated after the plants had reached the second-leaf stage, with a spore suspension poured on the soil.

In order to test the comparative efficiency of the two types of inoculum, 16 cans of soil were sterilized in the usual manner. Just prior to seeding with Kansas selection 9A, the contents of 21-day-old, 250 c. c. flask cultures of strain A and strain B were added to each of four cans and mixed thoroughly with the surface 4 inches of soil. The eight remaining cans were inoculated, four each with spore suspensions of the two strains, by pouring the suspension on the soil. The cans were kept in a greenhouse in which the temperature was held at 17° to 18° C. until the plants had reached the second-leaf stage, as it was feared that there might be a heavy loss from damping off if the young seedlings were exposed to higher temperatures with the soil so heavily infested. When the second-leaf stage was reached the cans were transferred to a chamber where the temperature varied from 25° to 30°. Observations were made in the usual manner, and the results are shown in Table 3.

TABLE 3.—Percentage of seedlings of Kansas selection 9A which developed infection in soil inoculated at time of planting with spore suspension or with mycelial mass of strains A and B of *Fusarium lycopersici*. Experiment 1

Strain	Percentage of plants dead and wilted, inoculated with—		Percentage of plants with blackened bundles, though apparently healthy, inoculated with—		Total percentage of plants affected, inoculated with—	
	Spore suspension <sup>a</sup>	Mycelial mass <sup>a</sup>	Spore suspension <sup>a</sup>	Mycelial mass <sup>a</sup>	Spore suspension <sup>a</sup>	Mycelial mass <sup>a</sup>
A.....	36	34	26	26	62	60
B.....	2	2	3	2	5	4

<sup>a</sup> Approximately 100 plants used in each trial.

This experiment was repeated using the same methods except that the amount of infested barley kernels applied to each can of soil was practically tripled. The spore suspension was applied as before so that each can of soil was inoculated with the conidia from one Petri-dish culture. The results are shown in Table 4.

TABLE 4.—Percentage of seedlings of Kansas selection 9A which developed infection in soil inoculated at time of planting with spore suspension or with mycelial mass of strains A and B of *Fusarium lycopersici*. Experiment 2

Strain	Percentage of plants dead and wilted, inoculated with—		Percentage of plants with blackened bundles, though apparently healthy, inoculated with—		Total percentage of plants affected, inoculated with—	
	Spore suspension <sup>a</sup>	Mycelial mass <sup>b</sup>	Spore suspension <sup>a</sup>	Mycelial mass <sup>b</sup>	Spore suspension <sup>a</sup>	Mycelial mass <sup>b</sup>
A.....	30	75	27	19	57	94
B.....	1	8	1	2	2	10

<sup>a</sup> Approximately 100 plants used in each trial.

<sup>b</sup> Approximately 150 plants used in each trial.

The results of the two experiments shown in Tables 3 and 4 indicate that the difference in pathogenicity is due to some factor or factors inherent in the two strains. Despite the fact that the inoculum of strain B was as plentiful as that of strain A, the latter was markedly the more virulent of the two. The amount of the inoculum evidently had an important bearing upon the rate of infection, as may be seen by comparing Tables 3 and 4. In the first experiment, the two types of inoculum were of equal efficiency. In the second experiment, the percentage infection induced by the spore suspension was comparable with that of the preceding trial, but that induced by the larger amount of mycelial inoculum was much higher. There is an additional possibility that the increased infection might have been partially due to the additional organic matter added to the soil when the larger amount of barley kernels was applied, as it has been shown (16) that the development of the disease is influenced by this factor. Whether this is due to improving the content of the soil as a medium for the development of the fungus, or to the weakening of the plants when grown in soils rich in crude fiber, has not been determined.

#### PATHOGENICITY OF SALTATION STRAINS

Although a great deal of progress has been made during the past few years on the isolation of new strains from saltation sectors, little has been done in testing the pathogenicity of the new isolations. Christensen (9) found that three of his new strains arising in this manner were less pathogenic than the cultures from which they were obtained, while two were more pathogenic. Christensen and Stakman (10) found that strains of *Ustilago zeae* isolated from saltation sectors varied in pathogenicity from the parent form, the new strains being less pathogenic in every case.

As the new saltation strains C and D possessed many characteristics, such as heavy sporulation and intense color production, that have been associated with high pathogenicity (34), their virulence was tested comparatively with that of strains A and B, standard methods of technic being employed. The experiment was concluded 51 days after the seed was planted, with results as indicated in Table 5.

TABLE 5.—*Pathogenicity of strains A and B compared with that of the strains (C and D) isolated from saltation sectors produced by strain B, to seedlings of Kansas selection 9A, grown in artificially inoculated soil held at temperatures of 25° to 30° C.; inoculum applied as seed was planted; approximately 100 plants used in each trial*

Strain	Shortest incubation period (days)	Percentage of plants dead or wilted	Percentage of plants with blackened bundles, though apparently healthy	Total percentage of plants infected
A.....	36	27	20	47
B.....	50	1	0	1
C.....	45	5	0	5
D.....	35	5	4	9

Another experiment, comparing the virulence of strains A, B, and D, was conducted along similar lines. Methods of administering the inoculum and watering the plants differed somewhat from those employed previously. The results corresponded with those obtained in the earlier experiment, the percentage infection of strains A, B, and D being 16, 3, and 9 per cent, respectively. Strain A proved less pathogenic than usual, due probably to the saturated condition of the soil (12).

These results show that the saltation strains, though more virulent than the parent strain B, are less pathogenic than strain A. This again demonstrates that virulence is not necessarily correlated with spore load—for the inoculum from strain D contained a far larger number of both macro and micro conidia than the inocula of the other strains tested—but is due rather to some characteristic inherent in the fungus itself.

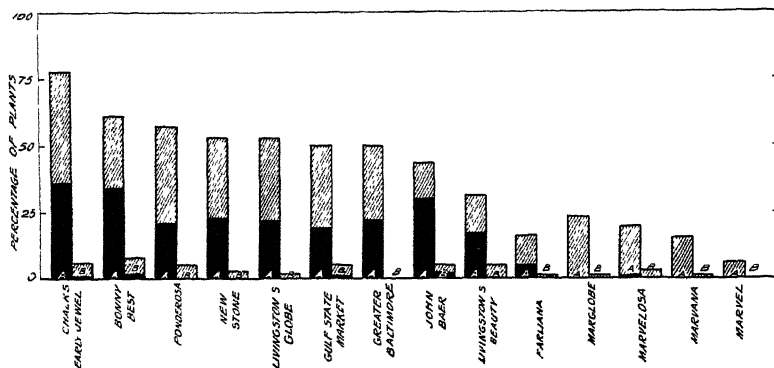


FIG. 3.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead or wilted, lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 1

#### PATHOGENICITY OF STRAINS A AND B TO SEEDLINGS OF DIFFERENT TOMATO VARIETIES UNDER CONDITIONS FAVORABLE FOR INFECTION

Breeding for disease resistance is rendered more complex by the existence of specialized races of the pathogene which vary in their pathogenicity toward different hosts. This is especially true when additional biologic forms exist that parasitize the varieties of the host first found to be resistant, as is true in the case of the two wheat rusts (23, 28), bean anthracnose (1, 2, 7, 19), and corn smut (10). To determine if a similar relationship existed between strains of *Fusarium lycopersici*, it was deemed advisable to test the virulence of strains A and B on a representative group of field and greenhouse varieties of tomatoes; also on those that have proved most successful in the several sections of the country in resisting wilt.

#### VARIETAL TEST NO. 1

In this experiment 10 commercial and 4 of the resistant varieties developed by Pritchard were tested. The soil, which had a  $P_H$  of 7.7, was prepared and inoculated in the usual manner. The results shown in Table 6 are represented graphically in Figure 3. Variations in the pathogenicity of the two strains to variety Greater Baltimore are illustrated in Figure 5, C, D.



TABLE 6.—*Pathogenicity of strains A and B of Fusarium lycopersici to different varieties of tomato grown in artificially inoculated soil held at 27° to 28° C., seed planted August 27, plants inoculated September 14, experiment concluded October 13, 1926, approximately 100 plants used in each trial*

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead or wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Chalk's Early Jewel.....	15	28	36	1	41	5	77	6
Bonny Best.....	19	28	34	2	28	6	62	8
Ponderosa.....	22	---	21	0	37	5	58	5
New Stone.....	20	---	23	0	30	3	53	3
Livingston's Globe.....	22	---	22	0	32	2	54	2
Gulf State Market.....	22	29	19	1	32	4	50	5
Greater Baltimore.....	22	---	22	0	28	0	51	0
John Baer.....	22	27	30	2	12	3	42	5
Livingston's Beauty.....	21	---	18	0	13	5	31	5
Earlana.....	22	---	5	0	11	1	16	1
Marglobe.....	---	---	0	0	23	1	23	1
Marvelosa.....	22	---	1	0	19	3	20	3
Marvana.....	---	---	0	0	16	1	16	1
Marvel.....	---	---	0	0	6	0	6	0

## VARIETAL TEST NO. 2

The soil, though prepared as before, had a slightly higher water-holding capacity. The  $P_H$  of the soil, which measured 7.6, was essentially the same as that in the other series. Commercial field varieties, English forcing varieties, and others selected for resistance by Pritchard, by Edgerton and Moreland, and by White were tested. The results are shown in Table 7 and represented graphically by Figure 4 and by illustrations in Figure 5, A, B.

TABLE 7.—*Pathogenicity of strains A and B of Fusarium lycopersici to different varieties of tomato grown in artificially inoculated soil held at 27° to 28°; seed planted September 29, plants inoculated October 24, experiment concluded November 22, 1926; approximately 100 plants used in each trial*

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Carter's Sunrise.....	25	31	31	2	11	1	16	4	58	7
Sterling Castle.....	20	35	30	3	3	0	16	4	49	7
Kanora 6.....	25	35	24	3	8	0	15	1	47	4
Norton.....	25	30	21	3	12	0	12	0	45	3
Kanora 11.....	26	29	30	7	6	3	7	3	45	13
Monumental.....	25	29	27	7	8	1	10	3	44	11
Ailsa Craig.....	33	---	19	2	6	0	7	10	32	12
Norduke 3.....	25	29	13	3	7	1	10	1	30	5
Kansas selection 240-1-1.....	27	35	19	0	3	1	7	0	29	1
Avon.....	---	---	11	5	5	2	11	2	27	9
Bide's Recruit.....	33	35	15	0	3	1	6	2	24	3
Louisiana Pink.....	27	35	5	0	4	1	15	1	24	2
Kansas selection 220-1-2.....	25	35	7	1	4	1	11	1	22	3
Kansas selection 205-3.....	25	---	7	0	9	0	5	1	21	1
Louisiana Red.....	32	---	4	0	3	0	12	0	19	0
Kansas selection 205-1-1.....	30	35	5	1	3	0	4	2	12	3

These two varietal tests demonstrated several significant facts. (1) None of the varieties tested is entirely immune to the attack of the more pathogenic strain of the wilt fungus. (2) Varieties that have proved highly resistant under field conditions may be quite susceptible when conditions for infection are more favorable. This was shown in an especially marked manner in the trials of Norton and Kanora 11. With few exceptions, Norton has been reported resistant under an extremely wide range of conditions. Kanora 11, when tested in comparison with other varieties in Kansas, has proved very resistant under field conditions (33). (3) At least part of the resistance shown by certain varieties may be attributed to the plant's ability to produce a partial crop in spite of the presence of the pathogene in the host tissues. This merely adds some additional evidence to similar conclusions advanced by Edgerton and Moreland (16) and recognized by those who have studied tomato wilt critically. (4) There is a marked difference in the virulence of the two strains. In all of the varieties studied, strain B at no time produced higher than 12 per cent infection and in several cases was unable to cause any infection whatsoever. (5) There was no evidence of reciprocal

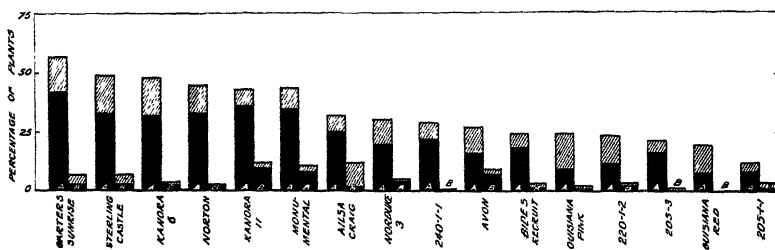


FIG. 4.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 2

resistance and susceptibility by different varieties to the two strains of the fungus. (6) Results obtained with some varieties did not agree with reports of the disease in the field. Livingston's Globe has been generally reported to be one of the most resistant of the commercial varieties, while the ability of Earliana to produce fair yields has been attributed to freedom from disease through early maturity (24). Under conditions favorable for infection, Livingston's Globe proved to be highly susceptible to strain A, while the evidence indicated that Earliana possesses factors other than early maturity which are of value in warding off the attack of the parasite. Greater Baltimore, regarded as one of the most susceptible varieties, while subject to the attack of strain A, seemed immune to strain B.

#### VARIETAL TEST NO. 3.

Because of the limited time left for the use of the temperature tanks, it was not possible to repeat the combined pathogenicity-varietal test with varieties other than those that had shown some characteristic that merited special consideration. Thus, Livingston's Globe, Earliana, and Greater Baltimore were tested a second time to determine if the results obtained before could be duplicated.

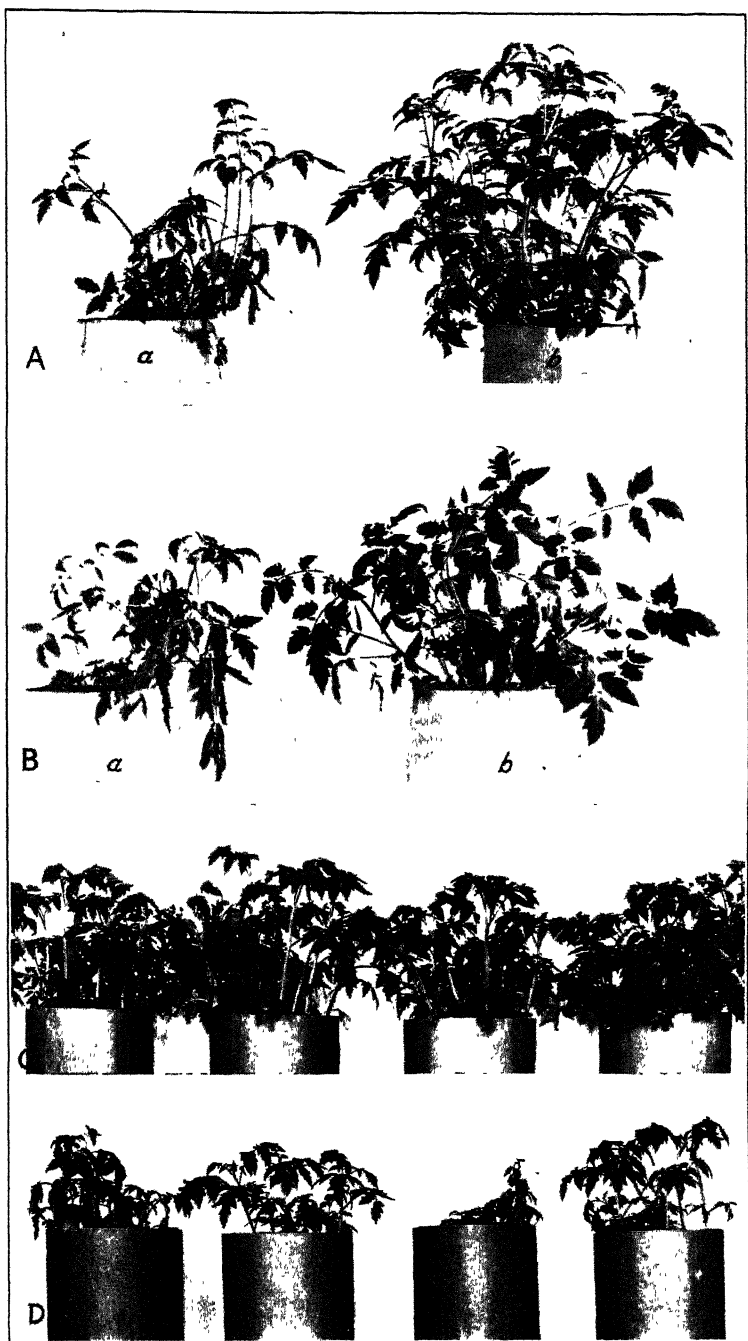


FIG. 5.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. A, to Norton: a, strain A; b, strain B. B, to Kanora 11: a, strain A; b, strain B. C, virulence of strain B to Greater Baltimore. D, virulence of strain A to Greater Baltimore.

The only departure from the methods used in the two former experiments was that the seed was allowed to germinate in a cool greenhouse and the young seedlings were held at temperatures ranging from 18° to 22° C. for three weeks before they were placed in the temperature tanks at 28°. The results of the experiment are shown in Table 8 and are represented graphically in Figure 6.



FIG. 6.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 3.

TABLE 8.—Pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato grown in artificially inoculated soil held at 28° C.; seed planted February 11, plants inoculated March 11; experiment concluded April 7, 1927: approximately 100 plants used in each trial

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Greater Baltimore.....	14	23	34	1	11	1	17	0	62	2
Earliana.....	19	27	26	0	5	2	38	1	69	3
Kansas selection 205-3.....	19	24	17	1	4	0	44	4	65	5
Livingston's Globe.....	19	27	18	0	4	1	23	1	45	3
Kansas selection 240-1-1.....	26	.....	3	0	2	0	48	1	53	1
Marvel.....	27	.....	0	0	2	0	50	0	52	0
Louisiana Red.....	22	.....	4	0	2	0	31	2	37	2
Marrana.....	19	.....	1	0	0	0	38	2	39	2

The results of this experiment were noteworthy in at least three respects. (1) The apparent constancy in the virulence of strain B and the greater pathogenicity of strain A were again demonstrated. (2) The ability of certain varieties to thrive for a time at least even

though the plants were heavily invaded, as shown by the number having blackened bundles, was demonstrated much more conclusively than in the earlier experiments. This was especially marked in the Kansas selection 240-1-1, in Marvana, and in Marvel, as the data show that the pathogene invaded a high percentage of the plants of these varieties without having displayed external indications of its presence. Plants of the variety Marvel, although infected to over 50 per cent in the strain A series, evidenced no more signs of a diseased condition externally than those treated with strain B in which

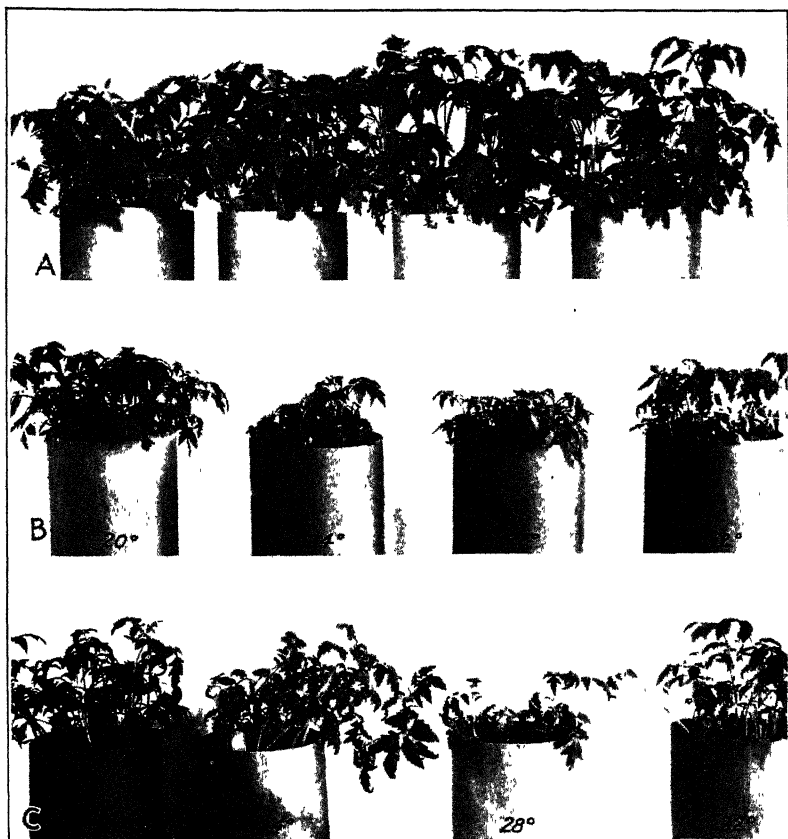


FIG. 7.—A.—Tolerance shown by Marvel seedlings to *Fusarium lycopersici*; 52 per cent of seedlings shown had blackened vascular bundles. B and C.—Seedlings of Kansas selection 9A, showing the relation of soil temperature to production of tomato wilt by a strain of *Fusarium lycopersici*; temperatures (°C.) as indicated

the infection was zero. (Fig. 7, A.) (3) With a single exception strain A proved to be more pathogenic than in the previous experiments. This might have been due to the fact that the strain had become more virulent while in culture, a development that would scarcely be expected since strain A had been stable and not at all inclined to saltate; or it might have been that the exposure of the plants to low temperatures prior to their inoculation had predisposed them to infection by the wilt fungus. The latter explanation would conform with results obtained in the study of certain other *Fusarium*

diseases and would seem to be the more likely (14, 30). This possibility is of such significance, from a practical as well as a scientific viewpoint, that it merits further consideration.

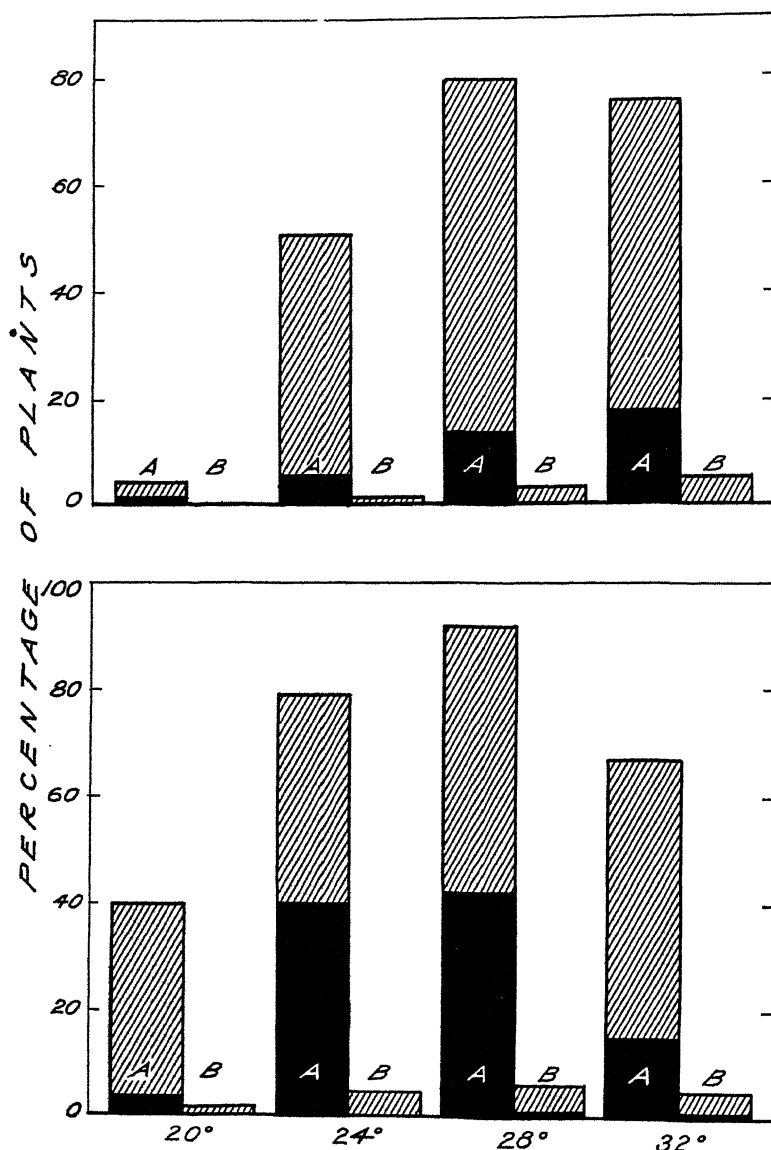


FIG. 8.—Seedlings of varieties Norton (upper) and Kansas selection 9A (lower), showing relation of soil temperature to wilt production. Plants grown in artificially inoculated soil. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy.

#### RELATION OF SOIL TEMPERATURE TO PATHOGENICITY

Considerable evidence has accumulated during the past few years, demonstrating the close relationship existing between soil temperature and the inception of certain diseases. In most of these experi-

ments, the relation of temperature to the development of the fungus on some solid culture medium, usually potato agar, has been determined and attempts have been made to correlate this with the relation of temperature to the development of the disease. Such relationships have coincided closely in the case of some of the diseases caused by vascular-invading *Fusaria* (31, 11, 16). The correlation is not so close in flax wilt (32), while in the case of corn root rot, in which the cortex is invaded, temperatures favoring the growth of the organism inhibit the development of the disease (13).

Clayton's investigations on the relation of temperature to infection by *Fusarium lycopersici* were limited to one strain of the fungus, to one variety of the tomato, and to the use of semimature plants only. His work was repeated in part by the writer for the following reasons: (1) To learn if infected seedlings react to different soil temperatures in a manner similar to that of older plants; (2) to ascertain if the results would be affected by the use of different host varieties grown directly from seed; (3) to learn more of the relation of temperature to resistance, and (4) to determine if the two strains of the pathogene show variations in susceptibility at different temperatures. White (34) found that strain B was much more virulent than A. His results were obtained with soil held at a lower temperature than that used in the writer's experiments. Because of this it was thought that his results, which were the reverse of those obtained in the present investigations, might have been due to the fact that strain B was favored by lower temperatures. If such were the case it would also have a practical bearing on greenhouse culture.

TABLE 9.—Percentage of plants of varieties Norton and Kansas selection 9A which showed infection when grown in soil of different temperatures inoculated with strains A and B of *Fusarium lycopersici*; seed planted January 10, plants inoculated February 6, experiment concluded March 9, 1927; approximately 100 plants used in each trial

Variety of tomato	Average soil temperature	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected *	
		Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Norton	20	26	-----	1	0	0	0	3	0	4	0
	24	24	-----	2	0	3	0	45	1	50	1
	27.5	19	-----	11	0	2	0	66	3	79	3
	32	16	-----	8	0	9	0	58	5	75	5
	20	25	-----	1	0	3	0	35	2	39	2
Kansas selection 9A	24.5	19	-----	29	0	11	0	39	5	79	5
	28	17	25	37	1	5	0	50	5	92	6
	32	18	28	10	0	5	1	52	3	67	4

\* A total of 476 plants, used as controls, showed no infection.

In order to determine the optimum temperature for the production of the disease on seedlings by each of the two strains, two experiments were conducted. Norton and Kansas selection 9A were selected for these trials in order that a resistant and a susceptible variety might be tested. Seed of these varieties from close pollinated plants was used. As it had been shown by Clayton (11) that his Indiana

strain of *Fusarium lycopersici* was pathogenic throughout the range 20° to 32° C., the temperatures were maintained at approximately 20, 24, 28, and 32 degrees. The soil was prepared and the inoculations were carried out as in the foregoing experiments. The results shown in Table 9 are represented graphically in Figure 8 and are illustrated by Figure 7, B and C.

The results of the studies with seedlings were in agreement with those previously obtained by Clayton (11) with more mature plants grown from cuttings. This was especially true of the strain A series in which infection was sufficiently heavy to give results of experimental value. Although the higher temperatures favored the production of wilt by strain B, the percentage of infection was so small that the variation in disease production at the different temperatures was not marked.

Temperatures favoring infection coincide fairly accurately with those promoting the radial expansion of the fungus on potato agar. Twenty-eight degrees was the optimum for disease production and for the radial extension of the fungus in culture. The fungus, on potato agar, grew almost as rapidly at 24° C., and the results with Kansas selection 9A show that it is possible for the disease to develop readily at this temperature. In the variety Norton, although there was a high percentage of bundle blackening at 24°, there was little wilt, temperatures above 28° C. apparently being necessary to the early development of the disease in a severe form. Results practically duplicating these were obtained in another experiment conducted in a similar manner. These data indicate that some varieties may be especially valuable in certain localities and during seasons in which soil temperature falls somewhat below 28°. Apparently some varieties may succumb to the disease at relatively low temperatures, while others retain their disease resistance.

#### DISCUSSION

If the differences in the two strains studied are characteristic of isolations from different parts of the country, *Fusarium lycopersici* is an extremely variable fungus. This variability may possibly be explained by the facility with which, when the fungus is subjected to favorable conditions, its nature may be changed by saltations. The possibility of the origin and dispersal of strains aggressively parasitic upon varieties now considered resistant, should not be underestimated. There is a remote possibility that variations in environmental conditions may be of importance, not alone in their effect upon the association of host and parasite, but also in inducing the fungus to change its inherent nature through general saltations.

Although the two strains exhibited marked differences in virulence, there was no case in which varieties which were resistant to the more virulent form of the fungus succumbed to the other strain. Hence the problem of breeding for resistance would not be so complicated as in the case of diseases like the cereal rusts and bean anthracnose. In addition, the strains of the tomato wilt fungus lack the stability characteristic of forms of the cereal rust fungi. Consequently, from the evidence obtained in these experiments, there would seem to be no value in attempting to establish varieties or forms of *Fusarium lycopersici*.



## SUMMARY

Comparative physiologic tests were made with two strains of *Fusarium lycopersici* Sacc., obtained from widely separated localities. The two strains were typical representatives of White's (34) dissimilar groups.

The two strains showed markedly different characteristics in culture. Strain A by several criteria proved to be very constant; strain B proved extremely variable, producing many saltations which differed from the parent form in appearance and in pathogenicity.

Without exception, strain A proved to be more pathogenic than strain B. The difference in pathogenicity could not be altered by regulating the soil temperature, increasing the spore concentration in the inoculum, or by using different types of inoculum.

A soil temperature of 28° C. proved the optimum for the production of wilt in seedlings of two varieties. A similar temperature proved to be the optimum for the lateral spread of colonies of the two strains on potato-dextrose agar.

The lack of stability displayed by different strains and the fact that variations in strain virulence were uniform on all host varieties tested, make it inadvisable, from the evidence obtained in these experiments, to establish varieties or forms of *Fusarium lycopersici*.

Resistance in certain varieties is apparently correlated with two factors. (1) The temperature range over which certain varieties are susceptible may be broader or may be different from that over which the more resistant varieties are subject to infection. This was indicated by preliminary trials in which Norton was more susceptible than Kansas selection 9A at 31° C., but was decidedly more resistant at 24° C. The divergence in results with resistant varieties obtained by workers in different localities may have been due to this characteristic. (2) Resistant varieties apparently possess certain physiological characteristics that enable them to tolerate the final attack of the pathogen in spite of its invasion of the host tissues. This was shown strikingly in the case of Marvel, which evidenced but 2 per cent wilting in spite of 52 per cent infection.

## LITERATURE CITED

- (1) BARRUS, M. F.  
1911. VARIATION OF VARIETIES OF BEANS IN THEIR SUSCEPTIBILITY TO ANTHRACNOSE. *Phytopathology* 1: [190]-195, illus.
- (2) ———  
1918. VARIETAL SUSCEPTIBILITY OF BEANS TO STRAINS OF COLLETOTRICHUM LINDEMUTHIANUM (SACC. & MAGN.) B. & C. *Phytopathology* 8: [589]-614, illus.
- (3) BRIERLEY, W. B.  
1920. ON A FORM OF BOTRYTIS CINEREA, WITH COLOURLESS SCLEROTIA. *Roy. Soc. [London], Phil. Trans. (B)* (210): 83-114, illus.
- (4) ———  
1922. DISCUSSION ON MUTATION OF SPECIES. *Brit. Med. Jour.* 3225: 722-726, illus.
- (5) BROWN, W.  
1923. EXPERIMENTS ON THE GROWTH OF FUNGI ON CULTURE MEDIA. *Ann. Bot. [London]* 37: [105]-129, illus.
- (6) ———  
1926. STUDIES IN THE GENUS FUSARIUM. IV. ON THE OCCURRENCE OF SALTATIONS. *Ann. Bot. [London]* 40: [223]-243, illus.

- (7) BURKHOLDER, W. H.  
1923. THE GAMMA STRAIN OF COLLETOTRICHUM LINDEMUTHIANUM (SACC. ET MAGN.) B. ET C. *Phytopathology* 13: [316]-323.
- (8) ———  
1925. VARIATIONS IN A MEMBER OF THE GENUS FUSARIUM GROWN IN CULTURE FOR A PERIOD OF FIVE YEARS. *Amer. Jour. Bot.* 12: 245-253.
- (9) CHRISTENSEN, J. J.  
1925. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN HELMINTHOSPORIUM SATIVUM. *Phytopathology* 15: [785]-795, illus.
- (10) ——— and STAKMAN, E. C.  
1926. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN USTILAGO ZEAE. *Phytopathology* 16: 979-999, illus.
- (11) CLAYTON, E. E.  
1923. THE RELATION OF TEMPERATURE TO THE FUSARIUM WILT OF THE TOMATO. *Amer. Jour. Bot.* 10: 71-88, illus.
- (12) ———  
1923. THE RELATION OF SOIL MOISTURE TO THE FUSARIUM WILT OF THE TOMATO. *Amer. Jour. Bot.* 10: 133-147, illus.
- (13) DICKSON, J. G.  
1923. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF THE SEEDLING-BLIGHT OF WHEAT AND CORN CAUSED BY GIBBERELLA SAUBINETII. *Jour. Agr. Research* 23: 837-870, illus.
- (14) ——— and HOLBERT, J. R.  
1926. SYMPOSIUM ON "THE PRESENT STATUS OF CORN IMPROVEMENT."  
3. THE INFLUENCE OF TEMPERATURE UPON THE METABOLISM AND EXPRESSION OF DISEASE RESISTANCE IN SELFED LINES OF CORN. *Jour. Amer. Soc. Agron.* 18: 314-322, illus.
- (15) EDGERTON, C. W.  
1918. A STUDY OF WILT RESISTANCE IN THE SEED-BED. *Phytopathology* 8: [5]-14, illus.
- (16) ——— and MORELAND, C. C.  
1920. TOMATO WILT. *La. Agr. Expt. Sta. Bul.* 174, 54 p., illus.
- (17) ——— and MORELAND, C. C.  
1921. TESTS OF THE WILT RESISTANCE OF DIFFERENT TOMATO VARIETIES. *La. Agr. Expt. Sta. Bul.* 184, 24 p., illus.
- (18) HAYMAKER, H. H.  
1928. RELATION OF TOXIC EXCRETORY PRODUCTS FROM TWO STRAINS OF FUSARIUM LYCOPERSICI SACC. TO TOMATO WILT. *Jour. Agr. Research* 36: 697-719, illus.
- (19) LEACH, J. G.  
1923. THE PARASITISM OF COLLETOTRICHUM LINDEMUTHIANUM. *Minn. Agr. Expt. Sta. Tech. Bul.* 14, 41 p., illus.
- (20) LEONIAN, L. H.  
1925. PHYSIOLOGICAL STUDIES ON THE GENUS PHYTOPHTHORA. *Amer. Jour. Bot.* 12: 444-498, illus.
- (21) ———  
1926. THE MORPHOLOGY AND THE PATHOGENICITY OF SOME PHYTOPHTHORA MUTATIONS. *Phytopathology* 16: 723-730, illus.
- (22) MCCLINTOCK, J. A.  
1920. TOMATO WILT. *Ga. Agr. Expt. Sta. Bul.* 138, p. [39]-49, illus.
- (23) MAINS, E. B., and JACKSON, H. S.  
1926. PHYSIOLOGIC SPECIALIZATION IN THE LEAF RUST OF WHEAT, PUCINIA TRITICINA ERIKSS. *Phytopathology* 16: [89]-120, illus.
- (24) PRITCHARD, F. J.  
1922. DEVELOPMENT OF WILT-RESISTANT TOMATOES. *U. S. Dept. Agr. Bul.* 1015, 18 p., illus.
- (25) SCOTT, I. T.  
1924. THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF FUSARIUM LYCOPERSICI AND ON TOMATO WILT. *Missouri Agr. Expt. Sta. Research Bul.* 64, 32 p., illus.
- (26) SHAPOVALOV, M., and LESLEY, J. W.  
1924. THE BEHAVIOR OF CERTAIN VARIETIES OF TOMATOES TOWARDS FUSARIUM-WILT INFECTION IN CALIFORNIA. *Phytopathology* 14: [188]-197, illus.

- (27) SHERWOOD, E. C.  
1923. HYDROGEN-ION CONCENTRATION AS RELATED TO THE FUSARIUM WILT OF TOMATO SEEDLINGS. *Amer. Jour. Bot.* 10: 537-553, illus.
- (28) STAKMAN, E. C., and PIEMEISEL, F. J.  
1917. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. *Jour. Agr. Research* 10: 429-496, illus.
- (29) STEVENS, F. L.  
1922. THE HELMINTHOSPORIUM FOOT-ROT OF WHEAT, WITH OBSERVATIONS ON THE MORPHOLOGY OF HELMINTHOSPORIUM AND ON THE OCCURRENCE OF SALTATION IN THE GENUS. *Ill. Nat. Hist. Survey Bul.* 14: [77]-185, illus.
- (30) TIMS, E. C.  
1926. THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS. *Jour. Agr. Research* 33: 971-992, illus.
- (31) TISDALE, W. B.  
1923. INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS. *Jour. Agr. Research* 24: 55-86, illus.
- (32) TISDALE, W. H.  
1917. FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. *Jour. Agr. Research* 11: 573-606, illus.
- (33) WHITE, R. P.  
1926. TOMATO WILT INVESTIGATIONS. *Kans. Agr. Expt. Sta. Tech. Bul.* 20, 32 p., illus.
- (34) ———  
1927. STUDIES ON TOMATO WILT CAUSED BY FUSARIUM LYCOPERSICI SACC. *Jour. Agr. Research* 34: 197-239, illus.

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# RELATION OF TOXIC EXCRETORY PRODUCTS FROM TWO STRAINS OF *FUSARIUM LYCOPERSICI* SACC. TO TOMATO WILT<sup>1</sup>

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## REVIEW OF LITERATURE

Of the several theories which have been advanced to explain how wilting is produced by vascular-invading pathogenes, White (26)<sup>3</sup> and Rosen (21) have mentioned five, viz, (1) the parasitism theory of Atkinson (1), (2) the bundle-plugging theory, first elaborated by Stewart (24) in bacterial diseases, and by Smith (23) in fungous diseases and developed further (4, 13, 22) to include obstruction of vessels by products of the pathogene; (3) the rootlet-destruction theory first advanced by Orton (16); (4) the embolism theory of Tochinai (25); and (5) the toxin theory, advanced by Hutchinson (13) and further elaborated by Goss (8), Brandes (6), and many others during the past decade. Neither White nor Rosen mentioned the theory propounded by Van der Lek (15), that the actual wilting was due to the invasion of the leaf parenchyma by the organism. Van der Lek arrived at this conclusion after proving that the cucumber leaf did not wilt despite the invasion of the petiole by the mycelium of *Verticillium* until the hyphae had progressed to the lamina.

The greater part of the work conducted relative to the toxin theory has dealt with the nature of the toxic principle, and important results have been obtained. Nearly all of the investigations (3, 4, 5, 8, 9, 19, 21, 26, 27, and that by Tims<sup>4</sup>) dealing with metabolic products in liquid cultures of fungi have demonstrated that the toxic materials are for the most part thermostable. The sole evidence to the contrary has been furnished by later work of Goss (10). Specific principles, however, isolated from the filtrate by precipitation with alcohol, primarily enzymic in nature, have been partially (4) or wholly (26) inactivated by boiling.

Conflicting evidence has been obtained concerning the volatility of the toxic principle. Barnum (3), Fahmy (9), and Young and Bennett (27) concluded that it was nonvolatile. White (26) and Rosen (21) found initial fractions of distillation toxic to tomato and cotton plants. Lathrop (14) found that *Fusarium cubense* produced a volatile aldehyde which he thought might possibly be toxic.

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 718.

<sup>4</sup> Tims, E. C. STUDIES ON THE *FUSARIUM* DISEASE OF CABBAGE. 1924. [Unpublished manuscript. Copy on file, Library, Univ. Wis., Madison.]

Likewise, there is a difference of opinion regarding the relation of reaction of the filtrate to toxicity. Young and Bennett (27) and Tochinali (25) concluded that an increase in  $P_n$  was correlated with increased toxicity. Apparently they did not take into consideration the increased toxicity due to aging (5). On the other hand, Fahmy (9) and Bisby (5), while noting that the medium in which the fungus was growing became more alkaline, thought this change of little importance as a direct factor in causing affected plants to wilt.

Some progress has been made in separating from the filtrate the component materials which are capable of producing effects similar to wilt. Picado (19), Bewley (4), and White (26), working with various fungi, isolated exo- and endo-enzymes which were capable of causing symptoms resembling the fungous wilt. Picado also isolated a thermostable substance which was much more toxic than the enzymic material. White, by separating the components of the filtrate by dialysis, obtained thermostable materials, crystalloidal in nature, that were somewhat toxic to tomato plants. He as well as Bewley found that the filtrate would not diffuse through the membranes of an uninjured root system and cause wilting. This observation suggested that the toxic principle was colloidal in nature. Barnum (3) and Rosen (21) obtained results contrary to these.

That noncolloidal materials might have some bearing upon wilting is suggested by the results of Haskell (11), Peltier (18), and White (26), who showed that symptoms similar to those produced by treatment with fungal extracts developed when plants were treated with dilute solutions of various organic acids. Fahmy (9) concluded that ammonia and oxalates, while present in the filtrate of *Fusarium solani*, occurred in quantities insufficient to cause wilting. Rosen (21) obtained evidence indicating that an inorganic salt, a nitrite, might be one of the toxic ingredients causing wilt of cotton.

Little has been done to date in attempting to correlate the action of the toxic materials from liquid cultures with the corresponding effect resulting from the action of the parasitic fungus on the host. It has been shown repeatedly (3, 5, 6, 9, 19, 26, 27, and by Tims<sup>5</sup>) that metabolic products from fungi lack the specificity displayed by the organisms from which they were derived, the filtrate from saprophytic forms having proved as toxic apparently as that from vascular *Fusaria*. On the other hand, Rosen (21) found that the toxicity of the filtrate of different fungi to cotton seedlings was in direct correlation with the taxonomic relationship of the organisms themselves. Also, White (26) showed that a variety of tomato resistant to *Fusarium lycopersici* showed greater resistance to the action of the filtrate of that fungus than did a susceptible variety. Tims<sup>5</sup> obtained some evidence indicating that *F. conglutinans* grown at a relatively low temperature produced materials of greater toxicity than when grown at temperatures approaching the optimum for the vegetative vigor of the fungus.

While investigations have dealt for the most part with the relation of products of the pathogene to wilting, the evidence presented by Haskell (11) and Overton (17) suggests also that decomposition products from dead host cells may play some part in the production of wilt.

<sup>5</sup> Tims, E. C. Op. cit.

## DEFINITION OF PROBLEM

The experiments on varietal response to two strains<sup>6</sup> of *Fusarium lycopersici* (12) indicated that their pathogenicity and the resistance of certain varieties might be explained to better advantage if the factor or factors responsible for wilting were known. Evident resistance of varieties like Marvel, which showed practically no sign of the disease despite the invasion of its tissues by the fungus, suggested that wilting was not caused primarily by bundle plugging. Evidence pointed to the fact that the products of the fungus must have some harmful effect upon the host tissues. Three leads were opened by the earlier experiments (12) which should be correlated with studies of the excretory products of the wilt fungus. Consequently, experiments were planned that would furnish some evidence on the following questions: (1) Are the excretory products of the two strains of equal toxicity? (2) Do different varieties of the host show variations in susceptibility toward the toxic products of the causal fungus that are comparable to their reaction toward the pathogene itself? (3) Is there any correlation between the effect of different temperatures upon the pathogenicity of the fungus and the toxicity of its metabolic products? If there is a positive correlation between pathogenicity of the organism and toxicity of its products, as expressed by these three relationships, some additional evidence might be gained incriminating toxic excretory products as the cause of wilting.

## EXPERIMENTAL METHODS

The methods of procuring the various toxic materials from liquid cultures of *Fusarium lycopersici* used in conducting the experiments were as given below.

The fungus was grown in liter flasks in 250 c. c. of Richard's solution<sup>7</sup> modified slightly by substituting dextrose for sucrose. The growth period varied in the different experiments, ranging usually from three to five weeks. At the close of this period the liquid portion of the culture was filtered through a double thickness of Carl, Schleicher, and Schüll's No. 590 filter paper in a Büchner funnel.<sup>8</sup> In those tests in which it was thought that wilting might not take place for several days the original filtrate was refiltered in a similar manner.

A double thickness of parchment paper was used as the selective membrane in those experiments in which the colloidal and crystalloidal materials were separated by dialysis. The material to be treated was dialyzed with distilled water for a period of 28 hours.

In an effort to obtain extracellular enzymes the filtrate from the cultures in Richard's solution was treated with an equal amount of 95 per cent alcohol. After a period of 24 hours most of the supernatant liquid was decanted or siphoned off, the precipitate was removed from the remainder by filtration, was rewashed with 95 per cent alcohol and again with ether, and was allowed to dry at laboratory temperature. It was then scraped off the filter paper and finally redissolved in an amount of distilled water equaling that of the original filtrate.

<sup>6</sup> The term "strains" as used in this paper refers simply to the two isolations of *Fusarium lycopersici* reported upon previously (12).

<sup>7</sup> The following formula was used: KNO<sub>3</sub>, 10 gm.; KH<sub>2</sub>PO<sub>4</sub>, 5 gm.; MgSO<sub>4</sub>, 2.5 gm.; FeCl<sub>3</sub>, 20 mgm.; dextrose, 50 gm.; and water, 1,000 c. c.

<sup>8</sup> Results of experiments proved that filtration through the finer-grained porcelain filters was unnecessary, as tomato seedlings placed in heavy spore suspensions of *Fusarium lycopersici* showed no signs of wilting after 48 hours, by which time seedlings placed in the toxic solutions had wilted completely. (Fig 1, A. B.)

The plants to be treated were hardened at 18° C. until the second or third leaf stage was reached and were then transplanted to pots and allowed to grow for three or four weeks at temperatures of 25°–32° C. By treating large numbers of seedlings in this way, stocky, vigorous plants of uniform size were obtained. These were cut below water before being placed in the different solutions.

Young plants thus treated usually wilt in a typical manner, the wilting process occurring in a definite sequence of stages. In order to indicate the relative toxicity of the products of the two strains, certain numerical values were assigned to the various stages of the wilting process from which an artificial "index of toxicity" could be recorded. The first definite sign of an abnormal condition is expressed by a flaccid condition of the plant. This is followed first by a drooping of the stem, and then after a rather protracted interval by a loss of turgor in the leaflet laminae. This condition is succeeded by the collapse of the leaflets. That this stage, rather than the initial drooping of the stem, more closely parallels the true wilt is suggested by the fact that the plant is seldom able to recover from this condition. After the leaflets collapse they first become water-soaked and finally dry and brittle. The only exception to this sequence of stages in the wilting process is found when exceptionally stocky, vigorous plants are placed in extracts of low toxicity, in which case the initial stem drooping is often replaced by a marginal wilting and an inward rolling of the laminae of the leaflets.

#### TOXICITY OF THE EXCRETORY PRODUCTS FROM STRAINS A AND B OF *FUSARIUM LYCOPERSICI* TO TOMATO PLANTS OF DIFFERENT VARIETIES IN DIFFERENT STAGES OF MATURITY

##### TOXICITY OF EXCRETORY PRODUCTS TO PLANTS IN SEEDLING STAGE

The results of the varietal tests (12) demonstrated that there was a distinct difference in pathogenicity between strains A and B and also that the several varieties of tomato tested varied markedly in susceptibility. In order to determine the relation that might exist between the virulence of the fungus and the toxicity of its products, an experiment was conducted in which the same varieties were treated with different products of the fungus.

In the first series 14 varieties were treated with three different types of material—(1) the filtrate, which contained all of the toxic principles; (2) a redissolved alcoholic precipitate, which preliminary tests showed to be primarily colloidal in nature; and (3) the diffusate of the dialyzed filtrate, which consisted of crystalloidal materials. Each flask containing 250 c. c. of Richards' solution was inoculated with 10 c. c. of a heavy spore suspension. One-half of the cultures were inoculated with strain A, the remainder with strain B. The cultures were 3 weeks old when the mycelial mat was removed by filtration. The colloidal and crystalloidal materials were obtained in the usual manner. Plants of the different varieties were grown in sterile soil from seed planted 40 days earlier. Each plant was pulled up by the roots and the stem was cut under water before it was placed in the solution. Sterile distilled water and Richards' solution were used for controls.

The first evidence of drooping was noticed in the plants subjected to Richards' solution. They showed a slight flagging within an hour after being placed in the extract. That this flagging was caused by



the concentration of the medium was indicated by the fact that the plants regained their turgidity some hours later, as the osmotic relation between the solution and the cell sap became more nearly balanced.

In this experiment the colloidal materials in the alcoholic precipitate were practically innocuous; so the data were not included with that of the remaining treatments. This lack of toxicity was probably due to the comparatively short length of time during which the fungus had been growing, and to the fact that all of the colloidal materials were not recovered in the precipitate. The results obtained with the filtrate and the crystalloidal materials of the diffusate are summarized in Table 1.

TABLE 1.—*Toxicity of the excretory products of strains A and B, obtained from the filtrate of a 3-weeks-old culture grown on modified Richards' solution, to seedlings of 14 varieties of tomato*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Nature of variety as demonstrated by seedling tests in greenhouse	Variety of tomato	Effect of filtrate						Effect of crystalloidal materials						Index of toxicity, sum of observations		
		Hours required to produce first stem drooping		Index of toxicity after 10 hours		Index of toxicity obtained from the sum of three observations, taken after 4, 10, and 22 hours		Hours required to produce complete brittling of leaves		Index of toxicity after 30 hours		Index of toxicity obtained from the sum of three observations				
		Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B			
Susceptible.	Chalk's Early Jewel.....	3	5	6	5	20	12	56	62	5	1	18	12	38	24	
	Bonny Best.....	10	10	6	6	20	18	62	62	6	6	18	18	33	36	
	Ponderosa.....	10	10	5	5	18	13	56	56	7	6	25	19	43	32	
	New Stone.....	10	22	6	0	14	8	62	62	5	5	17	18	31	26	
	Livingston's Globe.....	10	10	5	5	13	13	56	62	6	6	19	18	32	31	
	Gulf State Market.....	10	10	5	5	11	11	62	62	7	7	20	20	31	31	
	Greater Baltimore.....	9	10	6	5	19	18	48	56	7	6	27	19	46	37	
	John Baer.....	3	3	6	6	20	20	48	48	7	7	25	24	45	44	
Moderately resistant.	Earliana.....	10	22	6	0	14	8	48	62	6	6	26	19	40	27	
	Livingston's Beauty.....	22	22	5	5	13	11	62	62	6	6	18	18	31	29	
Resistant....	Marglobe.....	3 <sup>1</sup>	2	10	6	5	19	13	62	62	5	1	18	11	37	24
	Marvelosa.....	10	10	5	5	11	11	62	62	5	5	18	17	29	28	
	Marvana.....	22	22	0	0	8	6	56	56	5	5	18	18	26	24	
	Marvel.....	22	22	0	0	6	6	56	62	5	5	19	16	25	22	
Total.....				67	52	206	168			82	72	286	247	492	415	

In the second trial the 16 varieties tested in the second varietal-pathogenicity series (12) were exposed to the three types of toxic materials. The methods resembled those used in the first trial with two notable exceptions—(1) the strains of the fungus were allowed to grow in culture for five weeks instead of three and (2) the alcoholic precipitate was redissolved in an amount of water equaling but one-half that of the filtrate from which it was obtained. This provided a more concentrated solution, which in many cases caused initial stem drooping more rapidly than did the filtrate. Plants in the latter solution, however, wilted permanently more quickly in spite of its lighter concentration. The results are shown in Table 2.

TABLE 2.—*Toxicity of different excretory products from 5-weeks-old cultures of strains A and B grown on Richards' solution to the seedlings of 16 varieties of tomato*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Nature of variety as demonstrated by seedling tests in greenhouse	Variety of tomato	Effect of filtrate				Effect of products (mainly colloidal) in the alcoholic precipitate				Effect of crystalloidal products				Index of toxicity, sum of observations	
		Hours required to produce complete wilting	Index of toxicity from observations after 5.5 hours		Index of toxicity from observations	Hours required to produce complete wilting	Index of toxicity from observations after 2.5 hours		Index of toxicity from observations	Hours required to produce complete drooping	Index of toxicity after 24 hours		Index of toxicity from sum of four observations		
			Strain A	Strain B			Strain A	Strain B			Strain A	Strain B			
Susceptible	Carter's Sunrise	6½	7	31	6½	7	25	14	5	7	3	67	61	Strain A	Strain B
	Sterling Castle	20	7	14	24	5	22	8	1	3	2	38	50		
	Kanora 6	16	7	24	22	6	23	5½	1	15	3	64	37		
	Norton	20	2	15	20	2	19	2	1	1	2	41	36		
Moderately resistant	Kanora 11	20	2	19	20	2	8	26	3½	4	13	8	63	Strain A	Strain B
	Monumental	24	7	24	5½	6	26	6	7	5	3	37	37		
	Norduke 3	22	1	10	9	6	24	3½	2	2	3	38	34		
	Kansas selection 240-1-1	27	1	8	22	5	26	15	2	5	3	31	36		
Resistant	Avon	18	5	23	22	6	11	21	3½	2	2	41	49	Strain A	Strain B
	Bide's Recruit	20	2	19	14	6	26	20	2	2	3	38	39		
	Louisiana Pink	29	1	10	15	3	22	27	0	0	0	26	31		
	Kansas selection 220-1-2	24	1	8	14	3	21	31	9	2	3	33	33		
Total	Kansas selection 205-3	20	2	17	24	3	21	21	38½	22	2	6	35	Strain A	Strain B
	Louisiana Red	18	2	11	24	1	14	15	5½	2	2	54	50		
	Kansas selection 206-1-1	20	2	24	10	6	29	25	24	1	2	54	50		
			43	269	281		77	70	354		37	64	43		

The results of these two series of experiments show (1) that the excretory products of strain A are somewhat more toxic than those of strain B, and (2) that varieties which show decided resistance or susceptibility to the organism exhibit similar characteristics when subjected to the toxic materials excreted by the pathogene. This is shown much more distinctly in those cases in which wilting did not occur quickly, thus enabling gradations in toxicity to be manifested. In series 2, as later experiments proved, more accurate results would have been obtained had the growth period of the fungus been limited to three weeks or four at the most. When the growth period is extended past three or four weeks, toxic materials, possibly disintegration products, accumulate in such quantities that the plants succumb too quickly for the manifestation of distinct variations in resistance. Moreover, differences in toxicity are much more apparent when actually observed than when indicated by numerical values. (Fig. 1.)

The relation between the response of resistant and susceptible varieties to the toxic materials is even more evident. (Fig. 2.)

This is indicated by the results shown in Table 3, in which the index of toxicity of 10 resistant varieties is compared with that of 10 varieties notably susceptible to wilt. Tests duplicating those of series 1 and 2 were repeated with 8 varieties of tomato and similar results were obtained.

TABLE 3.—*Toxicity of the excretory products of strain A of Fusarium lycopersici to 10 resistant and 10 susceptible varieties of tomato* \*

Resistant varieties	Index of toxicity	Susceptible varieties	Index of toxicity
Marvel.....	25	Chalk's Early Jewel.....	38
Marvana.....	26	Bonny Best.....	38
Marvelosa.....	29	Ponderosa.....	43
Marglobe.....	37	New Stone.....	31
Livingston's Beauty.....	31	Greater Baltimore.....	46
Norduke 3.....	28	Carter's Sunrise.....	67
Louisiana Red.....	35	Sterling Castle.....	39
Louisiana Pink.....	28	Kansas selection 9A.....	64
Kansas selection 205-3.....	38	John Baer.....	45
Kansas selection 220-1-2.....	31	Monumental.....	63
Average.....	31	Average.....	47

\* Classification as to susceptibility obtained from reports from field and greenhouse trials.

#### TOXICITY OF EXCRETORY PRODUCTS TO PLANTS IN BLOSSOMING AND FRUIT-BEARING STAGE

The experiments reported in the preceding pages indicated that the methods first used were not of sufficient delicacy to obtain a fair comparison of the relative toxicity of the two strains or to determine the comparative susceptibility of different varieties to the excretory products of the fungus. To provide a test of greater accuracy, it was thought that modifications might be made—(1) In the plant to be treated, (2) in the material administered, (3) in the technic of introducing the extract into the plant, or (4) in the environment of the plant after treatment.

Rosen (21) has shown that the more mature cotton plants do not succumb so quickly to the excretory products of *Fusarium vasinfectum* as do the younger plants. This is in agreement with results obtained

with the tomato when treated with the toxic products of *F. lycopersici*. Hence it was thought that if older plants were used, wilting would not occur so quickly and more accurate comparisons might be made.



FIG. 1.—A—*a*, Effect of a heavy spore suspension of *Fusarium lycopersici* in water on Marvana seedlings; *b*, effect of the filtrate from a liquid culture of *Penicillium* spp. on Marvana seedlings. B—*a*, Effect of a heavy spore suspension of *F. lycopersici* in Richards' solution on Marvana seedlings; *b*, effect of the filtrate from a liquid culture of *F. lycopersici* on Marvana seedlings. C—Relative toxicity of filtrate from liquid cultures of virulent strain A (*a*) and nearly innocuous strain B (*b*) to Marvana seedlings

Under field conditions the tomato plant is apparently most susceptible to the attack of the wilt fungus after the first blossoms form. In order to simulate natural conditions more closely, a series of 10 experiments was conducted in which plants just beginning to blossom were treated. The means employed in the earlier experi-

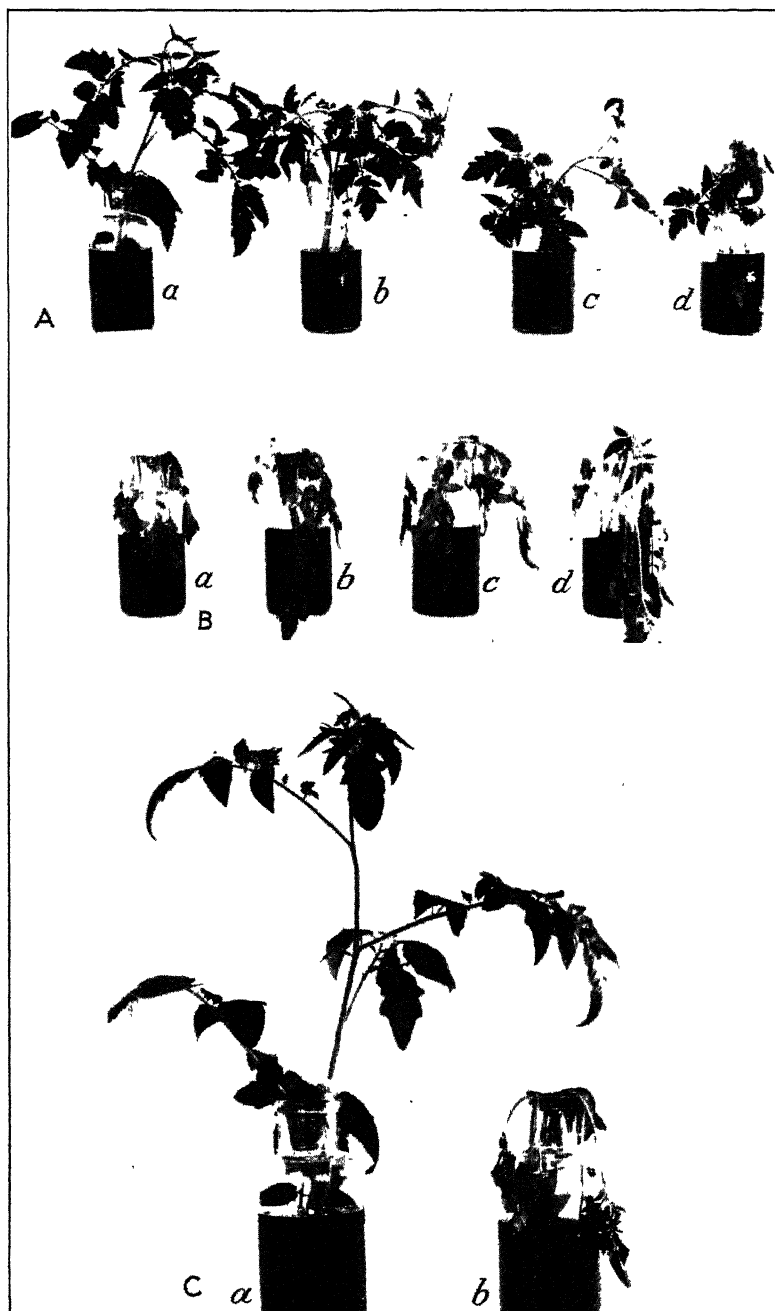


FIG. 2.—Relative susceptibility of seedlings of different varieties to the filtrate from cultures of *Fusarium lycopersici* grown in Richards' solution. A—Reaction of resistant varieties *a*, Kansas selection 240-1-1; *b*, Kansas selection 220-1-2; *c*, Louisiana Pink, and *d*, Louisiana Red. B—Reaction of susceptible varieties: *a*, Carter's Sunrise, *b*, Monumental, *c*, Kansas selection 9A, and *d*, Avon. C—*a*, Resistant variety Kansas selection 240-1-1 vs., *b*, susceptible variety Carter's Sunrise

ments in administering the extract seemed far removed from those that would naturally occur, so methods of technic were devised by which the extract might be introduced into the plants in a manner approximating infection by the fungus. The methods used, illustrated diagrammatically in Figure 3, follow.

#### EXPERIMENT 1

The method of stem excision below water was used. As plants had passed the seedling stage, the cut was made at the third node instead of at the soil line. The excised plants were placed in the solutions and were then left in the shade at laboratory temperature.



FIG. 3.—Diagrams illustrating methods of introducing toxic materials into tomato plants

That this treatment approached natural conditions somewhat more closely than that formerly employed was evidenced by the fact that the symptoms produced were more nearly like those in the field. Plants of this size when treated do not show drooping of the stem, so characteristic of seedlings treated in a similar fashion, the first indication of a diseased condition being the wilting and collapse of the laminae of the leaflets, followed by the drooping of petioles. While the initial stem drooping of the seedlings is usually closely correlated with the leaf wilting that follows, the latter seems to be much the more significant of the two.

The results of experiment 1 indicate that the products of strain A are more toxic than those of strain B, and that the variety Marvana, which is more resistant to the pathogene than the variety Monumental, is also more resistant to the metabolic products of the causal organism.

## EXPERIMENT 2

Experiment 2 was a duplicate of experiment 1, except that the filtrate administered was a week older and the plants treated were proportionately more mature than those used in the preceding trial. The results were essentially similar to those previously obtained.

## EXPERIMENT 3

Plants growing in 7-inch pots were used. The soil was washed away on one side until several of the larger lateral roots were exposed. These were severed below water and the roots to be treated were then introduced into vials containing 35 c. c. of the filtrate. The mouth of the vial was plugged with nonabsorbent cotton to prevent soil from entering. (Note fig. 3, A.)

The wilting which resulted from this treatment resembled that developing in the field. Wilting progressed from the lower to the higher leaves, affecting only those which were supplied by the roots treated. Thus a typical, unilateral wilt, which occurs so commonly under natural conditions, resulted. Individual leaves in some cases, as well as the plant as a whole, exhibited this one-sided wilting.

## EXPERIMENT 4

The plants were 24 weeks old. They had been grown at a temperature of approximately 19° C. in deep, rich soil in a greenhouse bench. They were especially sturdy, possessing heavy stems and extremely extensive root systems. The treatment was essentially the same as that of the preceding experiment, and the wilting that resulted was comparable to that under field conditions. The abnormal condition was first evidenced by a yellowing, then by wilting and brittling of the lower leaves, followed progressively by the same characteristics in the upper leaves. Following the collapse of leaflets, the petioles began to droop and eventually withered noticeably. Bundle discoloration became so pronounced that it could be detected easily by superficial examination. Repeated attempts to isolate an organism from the blackened bundles were unsuccessful in this and other experiments with larger plants.

The size and vigor of the plants and the comparatively low temperature at which they remained after the treatment resulted in an especially long incubation period, the first reliable indication of the diseased condition not being noticed for 10 days.

Contrary to expectation, it was found to be somewhat difficult to judge the relative toxicity of the products of the two strains, because differences were not so pronounced as often observed when seedlings were treated. A comparison was obtained by noting the number of leaves affected, the extent of their change, the proportion of the plants showing the diseased condition, etc. That this comparison might be as accurate as possible, plants of equal size and vigor were inoculated with the toxic products from each of the two strains. To reduce the possibility of biased judgment on the part of the writer, the readings were taken by disinterested parties, the same criteria for different stages of wilting being used in each case.

## EXPERIMENT 5

¶ The plants were removed from the 6-inch pots in which they had grown. The soil was washed from the lower half of the mass and the roots thus liberated were cut below water and introduced into a vial containing 35 c. c. of the filtrate. The entire mass of upper roots, together with the vial, was replaced in a larger pot partially filled with a sandy soil. Thus the treated plant received the fungal extract through the lower roots at the same time that the uninjured upper roots were functioning normally. (Fig. 3, B.)

## EXPERIMENT 6

¶ The plants were so treated that the lower roots and basal portion of the stem could be removed and cut below water without disturbing a mass of upper roots which had developed from subterranean portions of the stem. To obtain this result, the plants were treated as follows: Seedlings in the second-leaf stage, transplanted from flats, were thrust through the openings in 3-inch pots, and the roots and base of the stem were buried in soil in a 4-inch pot. As the plant increased in height, additional soil was placed around the stem in the upper pot in order to induce development of adventitious roots. The plants were kept at a low temperature (18.5° C.) to promote root development at the expense of aerial growth. At the end of 15 weeks the soil was washed from the roots in the lower pot, exposing the extreme base of the stem and the main roots which had developed from the lower portions of the stem. These roots and the base of the stem were then severed under water and placed in vials containing 35 c. c. of the filtrate. The treated plants were placed in the shade at laboratory temperature. (Fig. 3, E, F.)

## EXPERIMENT 7

Experiment 7 duplicated 6, except that but one variety was tested and the plants and cultures from which the filtrate was obtained were 1 week older.

## EXPERIMENT 8

When tomato seedlings in the fourth-leaf stage were transplanted to 7-inch pots they were so placed that the stems would angle off to one side to facilitate the washing out of dirt from the pot. At the time of treatment the soil was washed from one side of the mass of roots through the opening in the base of the pot. The stem, thus exposed, was cut below water and introduced into a vial containing 35 c. c. of filtrate. The vial was closed with a cork and the soil was replaced in the pot. (Fig. 3, C.) Wilting resulted in a relatively short time, as the plants were left in a high-temperature greenhouse, fully exposed to the sun.

## EXPERIMENTS 9 AND 10

In these two experiments the plants were treated in the same manner. The potted plants were placed in pot saucers filled with sand which was watered daily for a period of five weeks preceding treatment. As a consequence, a large mass of fibrous roots grew



through the opening in the pot into the sand in the saucer. After the sand had been washed off the roots were cut below water and immersed in a wide-mouthed vial containing 35 c. c. of the filtrate. After treatment the plant was watered from above, so that wilting would not be induced by a lack of soil moisture. (Fig. 3, D.)

Wilting developed in a much shorter time in experiment 9 than in experiment 10, due probably to the fact that a more susceptible variety was used; and also to the fact that somewhat larger root systems had developed, thus allowing the absorption of greater quantities of the filtrate before the vessels in the cut ends of the roots had become clogged.

The results of the 10 experiments are summarized in Table 4.

TABLE 4.—*Toxicity of the filtrate from cultures of strains A and B, grown in Richards' solution, to tomato plants of different varieties in the blossoming stage*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Experiment No.	Tomato variety	Age of plant	Number of plants used	Method by which extract was introduced into plants	Concentration of Richards' solution	Age of culture	Shortest incubation period		Index of toxicity	
							Strain A	Strain B	Strain A	Strain B
							Days	Hours	Hours	Hours
1	Marvana.....	11	6	Through stem cut off below water (at third node).	50	14	24	28	66	39
	Monumental.....	11	6	do.	50	14	20	20	73	61
2	Marvana.....	12	6	Through stem cut off below water (at third node).	50	21	9	10	37	32
	Monumental.....	12	6	do.	50	21	7	9	49	41
3	Gulf State Market.....	17	6	Through lateral roots cut off below water, potted plants.	100	41	38	44	28	14
	John Baer.....	12	12	Through lateral roots; plants in bench.	100	28	240	288	30	24
5	do.....	15	24	Through basal roots.	50	22	48	48	27	22
	do.....	15	4	do.	50	22	34	34	12	17
6	Louisiana Red.....	15	4	Through excised stem and roots; upper part of root system intact.	100	14	38	34	12	0
	do.....	15	4	do.	50	39	34	34	18	15
7	John Baer.....	16	26	do.	100	22	38	34	14	16
	Norton.....	15	12	Through excised stem and roots; upper root system partially intact.	100	22	16	17	54	41
8	do.....	15	12	do.	100	24	22	22	22	18
	John Baer.....	17	32	Through excised stem and roots; upper root system partially intact.	100	20	36	40	65	41
10	Kanora 11.....	15	12	Through roots projecting through base of pot.	100	35	120	192	50	38
	do.....	15	12	do.	100	35	120	192	50	38
Total.....			164						557	419

The results from this set of experiments correspond closely to those obtained with seedlings subjected to conditions favoring extremely rapid wilting. It was found that for purposes of comparison between strain behavior, or the response of different varieties to the toxic materials, the method employed in experiments 1 and 2 was as satisfactory as the others and involved less labor.

For purposes of strain comparison, the best results were obtained when excised stems of more mature plants of a resistant variety were

placed in the filtrate of 2-weeks-old cultures grown in 50 per cent Richards' solution and left in the shade at a temperature of 22° C.

The introduction of the toxic materials through the roots of plants is a slight departure from the more artificial system employed in practically all previous investigations. There is a marked resemblance between the symptoms of seedlings affected with *Fusarium* wilt and those of young plants which have absorbed toxic materials from fungal cultures. This resemblance is more pronounced when the symptoms of true wilt under field conditions are compared with those developing from the introduction of filtrate into the roots of fairly mature plants. This was shown in an especially marked degree in experiments 4, 9, and 10.

#### RELATION OF TEMPERATURE TO TOXICITY OF EXCRETORY PRODUCTS OF STRAINS A AND B OF *FUSARIUM LYCOPERSICI*

Repeated studies have been made wherein the ability of certain fungi to cause disease at different temperatures has been compared with their growth rates at those temperatures on some solid-culture medium. When the temperature which favored disease production corresponded to that at which the fungus made its most rapid radial expansion it was assumed that the development of the disease was primarily due to the fact that conditions were favorable for the growth of the fungus. On the contrary, when the two failed to correspond it was usually assumed that disease development was due to some weakened condition of the host which would promote infection. It seemed to the writer in the case of the wilt diseases, at least, that a temperature which might be ideal for the radial spread of a fungal colony on solid media might not be the one at which the fungus would be most pathogenic. It was thought that it might be of value to study the relation of temperature to the production of wilting by toxic fungal excretions. It seemed possible that a correlation in temperature relationships between the optima for disease development and the production of wilting by the toxic materials might be of greater significance than a correlation between temperatures favoring disease development and the lateral spread of the fungus.

It was suggested by Richards (20) that there might be two possible explanations for the maximum development of *Rhizoctonia* cankers on the potato, pea, and bean at temperatures below those which favored lateral spread of the fungus on solid-culture media—(1) that the pathogene might be inhibited by its own metabolic products at the higher temperatures, as suggested originally by Balls (2), or (2) that the fungus might secrete enzymes at the lower temperature which would be influential in causing a diseased condition. Richards presented no experimental evidence to support either suggestion. Aside from these observations, the writer is unaware of any reference in the literature in which it has been suggested that temperatures other than those which are the optimum for lateral mycelial expansion in culture might be the ones best adapted to the secretion of toxic materials directly responsible for the instigation of the disease.

In addition to securing further evidence regarding the relation of temperature to the production of the disease, it was thought that some temperature combination might be found that would provide a

favorable test for (1) the comparative toxicity of the excretory products of strains A and B, and (2) the comparative reaction of resistant and susceptible varieties of tomato to the wilt-producing factor. The following possibilities were considered: (1) That the temperature at which the plant was exposed when subjected to the toxic materials might be the one of greatest importance; (2) that the temperature at which the fungus was grown and at which the toxic materials were produced might be the critical one; (3) that a study in which the two factors mentioned were combined might provide evidence of the greatest value.

RELATION OF TEMPERATURE AT WHICH PLANTS WERE TREATED TO TOXICITY OF PRODUCTS OF STRAINS A AND B

The filtrate of 21-day-old cultures of strains A and B grown in Richards' solution at laboratory temperature (21°–22° C.) was obtained by the usual method, and 50 c. c. were placed in each of 48 flasks. The different portions of the filtrate were then placed in constant temperature chambers in which the temperatures ranged from 12° to 32° C. Two hours later seedlings of the varieties Stone and John Baer were cut under water and the stems inserted in the extract in the flasks. The results are shown in Table 5. Similar results were obtained when the experiment was repeated.

TABLE 5.—*Production of wilt at different temperatures by the filtrate of 3-weeks-old cultures of strains A and B of Fusarium lycopersici grown at 22° C.*

[Index of toxicity based on the following values: Plant turgid=0, stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Temperature at which toxicity of filtrate was tested (° C.)	Relative humidity in the different chambers	Hours required to produce complete leaf brittling (John Baer variety)		Index of toxicity after 24 hours; sum of observations (John Baer and Stone varieties)	
		Strain A	Strain B	Strain A	Strain B
12	69	-----	-----	0	1
16	61	-----	-----	20	18
20	60	30	-----	43	23
24	74	48	48	37	25
28	60	24	48	54	40
32	85	24	30	55	48

The results of this experiment indicate that both temperature and humidity play important rôles in the production of wilt by the toxic materials formed by the growth of the fungus in liquid culture. The conditions favoring rapid transpiration, viz, high temperature and low relative humidity, apparently cause a rapid absorption of the toxic materials and are thus best adapted to the production of wilt in the plants. Additional experiments demonstrated that humidity, as well as temperature, is an important factor in the development of wilting. Plants in the 12° chamber, in which the relative humidity was 60, wilted more quickly than those exposed to a temperature of 16° C. and a relative humidity somewhat higher. Similarly, plants held in a greenhouse at an average temperature of 26° and a relative humidity of 42 wilted in practically the same time as plants subjected

to a temperature of 32° and relative humidity of 80 and much more quickly than those kept at a temperature of 28° and a relative humidity of 88.

Differences in toxicity between strains A and B were more pronounced at the higher temperatures favoring wilt production than at the lower temperatures.

#### RELATION OF TEMPERATURE TO PRODUCTION OF TOXIC MATERIALS BY STRAINS A AND B

To determine the relation of temperature to the production of toxic materials, six experiments were conducted in which different varieties of plants and the filtrates from 2, 3, and 4 weeks old cultures were employed. The cultures were grown at 12°, 16°, 20°, 24°, 28°, and 31° C.;

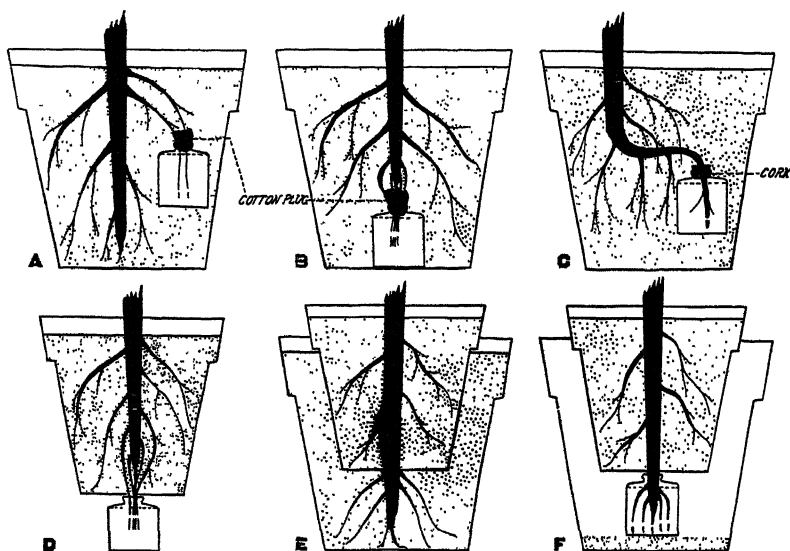


FIG. 4.—Relation of temperature to the production of toxic materials by *Fusarium lycopersici* grown in Richards' solution. A.—Seedlings of Kansas selection 9A in filtrate of liquid cultures grown at the following temperatures (left to right): 20°, 24°, 28°, 30° C. B.—Seedlings of Marvana in filtrate of cultures grown at the following temperatures (left to right): 12°, 16°, 20°, 24°, 28°, 31° C.

and 31° C.; the mycelial mat was removed by filtration in the usual manner and the stems of potted seedlings, cut under water, were inserted in the filtrate. In the first experiment in which 7-weeks-old Marvana and Monumental seedlings were treated, the plants, after insertion in the solutions, were placed in a high-temperature greenhouse at midday fully exposed to the sun. After a period of 60 minutes all of the plants of the variety Monumental showed a definite stem drooping and initial leaf flagging. This was especially marked in those plants in the filtrate of both strains that were grown at temperatures of 20° and above. At the same time all of the plants of the variety Marvana were erect and turgid, with the exception of those in the filtrate of cultures grown at 28°. In the solution of both strains obtained from cultures grown at 28° stems of the plants had drooped completely, so that they resembled those of the susceptible variety Monumental. (Fig. 4, B.) Collapse of the leaflets, which is probably more indicative of true wilt than stem drooping, also

occurred more quickly in plants subjected to filtrate produced at 28° than in those inserted in filtrates produced at other temperatures. This was shown more conclusively in additional experiments in which the plants, after having been cut below water, were placed in the filtrate from younger cultures and were then kept in the shade. (Fig. 4, A.) In a later experiment duplicating this one set of plants was placed in the filtrate of a 28° culture, previously cooled to 16°, and left at that temperature. Meanwhile the plants in the extracts produced at the various temperatures were exposed to an atmospheric temperature of 26° and a low relative humidity. The first plants to droop and wilt were those in the filtrate produced at 28° and held at the higher temperature. The plants next wilting were those in the 28° filtrate in the 16° chamber. Wilting in the plants exposed to 26° in the extracts produced at the other temperatures followed. The results of this experiment are summarized in Table 6.

TABLE 6.—*Production of wilting in tomato seedlings at 22° C. by the filtrate of cultures of strains A and B of Fusarium lycopersici grown in Richards' solution at different temperatures for 28 days*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Temperature at which fungus was grown (°C.)	Index of toxicity				
	By Maivana and Marvel (varieties resistant to the pathogene)		By Monumental, Stone, and Kansas selection 9A (varieties susceptible to the pathogene)		Total
	Strain A	Strain B	Strain A	Strain B	
12	10	12	19	19	60
16	12	12	58	42	124
20	39	27	77	67	210
24	53	29	84	68	234
28	86	85	123	113	407
31	35	39	63	64	201
Total--	235	204	424	373	1,236

These results indicate a definite correlation between temperatures favoring the inception of the disease and the production of materials toxic to plants. There is also a positive correlation between the lateral spread of the fungous colony on solid-culture media and the production of toxic materials. In this connection interesting observations were made as to the condition of the filtrates when grown at different temperatures. The color and density of the liquid were directly comparable to the effects of the different extracts. The filtrate from the 28° C. culture was far darker and more dense than that produced at the other temperatures. The extract obtained from the 24° culture was noticeably darker than those from the 20° and 31° cultures, which were of practically equal density. The filtrates from the cultures growing at 12° and 16° were nearly as clear as the original Richards' solution, which was used as a control. The thickness of the mycelial mat was not so noticeable in the 28° culture as in that grown at 24°, due to the fact that autolysis in the former had pro-

gressed to a more advanced stage. The greater density and deeper color of the filtrates produced at the temperatures best adapted to wilting were due apparently to the accumulation of larger amounts of colloidal material. This conformed with the idea that the metabolic material of highest toxicity is colloidal in nature.

In this series of experiments comparative measurements indicating relative toxicity were made with greater accuracy because the use of younger cultures and the treatment of the plants after the extract was administered provided tests of greater delicacy than those previously employed. The data in Table 6 indicate that the plants of varieties susceptible to the pathogene wilted much more quickly than resistant varieties. In addition it is evident that the metabolic products of the virulent strain A are more toxic than those of the nearly innocuous strain B to susceptible and resistant varieties alike. This was not so marked when plants were treated with material produced at the extreme temperatures, 12° and 31° C. In considering the equal toxicity of the two strains at 31° it may be remembered that strain B made a more rapid radial growth on potato agar than strain A at temperatures above 30°.

RELATION OF TEMPERATURE AT WHICH PLANTS WERE TREATED TO TOXICITY OF PRODUCTS OF STRAINS A AND B FORMED AT DIFFERENT TEMPERATURES

As in the previous experiment, the toxic materials were produced in cultures kept at different temperatures for 35 days. Two hours before the tomato seedlings were treated filtrates produced at the various temperatures were placed in the chambers in which the cultures had grown. Two-months-old Marvana and Monumental seedlings were used for the test.

TABLE 7.—*Production of wilting in tomato seedlings by the filtrate of 5-weeks-old liquid cultures of strains A and B of Fusarium lycopersici at the same temperatures at which the fungus was grown*

[Index of toxicity based on the following scale of values: Plant turgid=0; stem drooping=2; leaf collapse=6; water-soaked leaves=8; leaves dry and brittle=10]

Temperature at which fungus was grown and at which filtrate was held while tested for toxicity (°C.)	Relative humidity of the chambers	Index of toxicity after 24 hours <sup>a</sup>		
		Strain A	Strain B	Total
12	77	8	8	16
16	75	7	7	14
20	44	28	26	54
24	85	27	24	51
28	85	33	32	65
31	85	32	32	64

<sup>a</sup> Results of four trials.

The two previous experiments had shown (1) that if the tomato seedlings were treated with toxic materials produced at the same temperature wilting would be accelerated by a rise in temperature, and (2) that a greater quantity of toxic products, or materials of a higher degree of toxicity, were produced at 28° C. than at the other

temperatures. By producing wilt at the same temperature at which the toxic materials had been formed it was hoped that some information might be obtained as to which temperature factor was the more important—the one influencing the formation of deleterious metabolic products of the fungus or the one that would stimulate wilting after the toxic materials had been administered. The results of the first experiment are shown in Table 7. Experiments duplicating the one recorded produced results essentially similar to those indicated.

From these data it would seem that final wilting is influenced (1) by the temperature which is best adapted to the pathogene and (2) by those temperatures and atmospheric conditions which apparently cause some lack of physiological balance in the treated plant. Practically the same index of toxicity was recorded for the 28°–28° C. and 31°–31° combinations in all of the experiments conducted. The influence of humidity is evidenced again in the apparently greater toxicity of the 20°–20° combination as compared with the 24°–24° combination.

#### DISCUSSION

If wilting in nature is caused by fungal excretory products it would seem from the results of these three experiments that optimal conditions for the development of the disease would result from that combination of environmental factors best promoting the production of toxic materials by the fungus and at the same time predisposing the plant to natural wilting.

Theoretically, an ideal combination would be a temperature of 28° C. for the greater part of the day, during which time toxic materials would be produced in greatest abundance, followed by periods of higher temperature in which the plant would be more inclined to wilt from the increased rate of transpiration. This would agree with the findings of Clayton (?), who has presented evidence to prove that ideal conditions for the development of wilt are those that combine a temperature of 28° during the greater part of the day with intermittent periods of strong sunlight and higher temperatures. It would also agree with the results of the temperature tests conducted by Clayton as well as the writer, in which it was found that temperatures in the neighborhood of 31°–32° were more favorable for wilt production than a temperature of 24°, in spite of the fact that the latter is more conducive both to the radial spread of the fungus on solid media and to the production of toxic materials.

The most significant fact obtained from the three experiments was that the temperature which is ideal for the development of the disease was the most favorable for the production of wilting. Whether the greater toxicity of the filtrate produced at this temperature is due to a quantitative or a qualitative relationship is undetermined. The greater accumulation of materials, apparently colloidal in nature, suggests that the quantitative factor is of great importance.

#### STATISTICAL RECORD INDICATING RELATIVE TOXICITY OF EXCRETORY PRODUCTS OF STRAINS A AND B

In most of the experiments dealing with the relation of toxic fungal excretions to wilt the products of both strains were used in order to obtain a comparison of their ability to cause wilting. In the various

experiments conducted a total of 3,316 observations were recorded involving 1,658 comparisons of the relative toxicity of the products of the two strains. The results of these observations may be noted by the statistical record in Table 8.

TABLE 8.—Statistical record indicating roughly the relative toxicity of the excretory products of strains A and B of *Fusarium lycopersici*

Experiment No.	Number of comparisons	Strain A more toxic	Strains A and B of approximately equal toxicity	Strain B more toxic
1 (first varietal test).....	192	55	129	8
2 (second varietal test).....	425	68	325	32
3 (third varietal test).....	163	37	83	43
4 (10 experiments with plants in the blossoming stage).....	206	110	68	28
5 (temperature experiments).....	672	192	380	100
Total.....	1,658	462	985	211

The data in Table 8 show that in the majority of cases excretory products from virulent strains are apparently no more toxic to plants than those from practically innocuous strains. When there is an apparent difference, however, the products from the more virulent strain are usually the more toxic. This is indicated more strikingly in experiments in which more delicate tests were made. Thus in experiments 2, 3, and 5, listed in Table 8, in which 5 and 6 weeks old cultures were used, the results do not indicate such distinct variations in toxicity as do those of experiments 1 and 4, in which the methods were improved by the use of younger cultures and older plants.

In later experiments, in which the cultures were grown for two or three weeks in 50 per cent Richards' solution, the products of strain A were practically always more toxic than those of strain B.

#### SUMMARY AND CONCLUSIONS

A definite correlation was found to exist between the pathogenicity of *Fusarium lycopersici* and the toxicity of its metabolic products. This correlation was expressed in (1) similarity of symptoms (fig. 5), (2) the relation of different strains of the pathogene to wilting, (3) the similar reaction of susceptible and resistant varieties to the pathogene and to its toxic products, and (4) the relation of temperature to wilting. These results provide additional evidence to show that final wilting is caused, at least in part, by toxic materials liberated by the fungus. They also suggest that resistance is influenced to some degree by physiological factors.

When tomato plants in the blossoming stage were treated with the filtrate from liquid cultures of strains A and B of *Fusarium lycopersici* symptoms similar to those occurring in older plants in the field resulted.

Varieties that were resistant to the fungus itself were likewise more resistant to the excretory products of the fungus grown in culture than were varieties strongly susceptible to the fungus. Similarly, the metabolic products of the more pathogenic strain A proved to be more



toxic on the whole than those liberated by the comparatively innocuous strain B.

Temperature and relative humidity were found to be important factors in causing wilting in plants inserted in fungal extracts. Plants subjected to toxic materials produced under similar conditions wilted more quickly the higher the temperature and the lower the atmospheric humidity.

The excretory products from cultures grown at 28° C. proved to be more toxic than those produced at other temperatures. Plants placed in the toxic materials produced at 28° and exposed to a temperature of 28° wilted in practically the same time as those with stems submerged in filtrate produced at 31° and subjected to a temperature of 31°.



FIG. 5.—Wilting of a tomato plant (a) caused by the insertion of filtrate from a liquid culture of *Fusarium lycopersici* into the root system

The predisposition of the tomato to wilt at higher temperatures is apparently due to a combination of factors: (1) The vigor of the pathogene at temperatures ranging from 24° to 30° C.; (2) the fact (which is probably of greater importance) that at those temperatures the fungus produces excretory materials of greatest toxicity to the host plant; (3) at temperatures exceeding 30° the unbalanced physiological condition of the host plant renders it more subject to the action of the toxic products. This is probably a factor of great importance, since temperatures of 30° to 32° are more conducive to wilt production by the pathogene than those around 24°, in spite of the fact that the fungus grows more luxuriantly and produces materials of greater toxicity at the latter temperature.

## LITERATURE CITED

- (1) ATKINSON, G. F.  
1892. SOME DISEASES OF COTTON. Ala. Agr. Expt. Sta. Bul. 41, 65 p., illus.
- (2) BALLS, W. L.  
1908. TEMPERATURE AND GROWTH. Ann. Bot. [London] 22: [557]-591, illus.
- (3) BARNUM, C. C.  
1924. THE PRODUCTION OF SUBSTANCES TOXIC TO PLANTS BY *PENICILLIUM EXPANSUM* LINK. Phytopathology 14: [238]-243, illus.
- (4) BEWLEY, W. F.  
1922. "SLEEPY DISEASE" OF THE TOMATO. Ann. Appl. Biol. 9: 116-134, illus.
- (5) BISBY, G. R.  
1919. STUDIES ON *FUSARIUM* DISEASES OF POTATOES AND TRUCK CROPS IN MINNESOTA. Minn. Agr. Expt. Sta. Bul. 181, 58 p., illus.
- (6) BRANDES, E. W.  
1919. BANANA WILT. Phytopathology 9: [339]-389, illus.
- (7) CLAYTON, E. E.  
1923. THE RELATION OF TEMPERATURE TO THE *FUSARIUM* WILT OF THE TOMATO. Amer. Jour. Bot. 10: 71-88, illus.
- (8) COONS, G. H.  
1916. POTATO WILT. Mich. Agr. Expt. Sta. Ann. Rpt. 29: 269-270.
- (9) FAHMY, T.  
1923. THE PRODUCTION BY *FUSARIUM SOLANI* OF A TOXIC EXCRETORY SUBSTANCE CAPABLE OF CAUSING WILTING IN PLANTS. Phytopathology 13: [543]-550.
- (10) GOSS, R. W.  
1924. POTATO WILT AND STEM-END ROT CAUSED BY *FUSARIUM EUMARTII*. Nebr. Agr. Expt. Sta. Research Bul. 27, 83 p., illus.
- (11) HASKELL, R. J.  
1919. *FUSARIUM* WILT OF POTATO IN THE HUDSON RIVER VALLEY, NEW YORK. Phytopathology 9: [223]-260, illus.
- (12) HAYMAKER, H. H.  
1928. PATHOGENICITY OF TWO STRAINS OF THE TOMATO-WILT FUNGUS, *FUSARIUM LYCOPERSICI*, SACC. Jour. Agr. Research 36: 675-695.
- (13) HUTCHINSON, C. M.  
1913. RANGPUR TOBACCO WILT. India Dept. Agr. Mem., Bact. Ser. 1: [67]-83, illus.
- (14) LATHROP, E. C.  
1917. THE GENERATION OF ALDEHYDES BY *FUSARIUM CUBENSE*. Phytopathology 7: 14-16.
- (15) LEK, H. A. A., VAN DER  
1918. ONDERZOEKINGEN OVER TRACHEOMYCOSEN: DE VERTICILLIOSE VAN DEN KOMKOMMER. Meded. Landbouwhoogeschool. [Wageningen] 15: 1-45, illus. [French résumé, p. [37]-43.]
- (16) ORTON, W. A.  
1902. THE WILT DISEASE OF THE COWPEA AND ITS CONTROL. U. S. Dept. Agr., Bur. Plant Indus. Bul. 17: 9-22, illus.
- (17) OVERTON, J. B.  
1911. STUDIES ON THE RELATION OF THE LIVING CELLS TO TRANSPIRATION AND SAP-FLOW IN *CYPERUS*. I-II. Bot. Gaz. 51: 28-63, 102-120, illus.
- (18) PELTIER, G. L.  
1912. A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC *BOTRYTIS* ON PEPPER AND LETTUCE. Missouri Bot. Gard. Ann. Rpt. 23: 41-74, illus.
- (19) PICADO, C.  
1923. SUR L'ACTION À DISTANCE DES CHAMPIGNONS PHYTOPATHOGÈNES. Cong. Path. Vég. Strasbourg, p. 28-34. Strasbourg.
- (20) RICHARDS, B. L.  
1923. SOIL TEMPERATURE AS A FACTOR AFFECTING THE PATHOGENICITY OF *CORTICIUM VAGUM* ON THE PEA AND THE BEAN. Jour. Agr. Research 25: 431-450, illus.

- (21) ROSEN, H. R.  
1926. EFFORTS TO DETERMINE THE MEANS BY WHICH THE COTTON-WILT FUNGUS, *FUSARIUM VASINFECTIONIS*, INDUCES WILTING. *Jour. Agr. Research* 33: 1143-1162, illus.
- (22) SCHIKORRA, G.  
1906. I. *FUSARIUM-KRANKHEITEN DER LEGUMINOSEN*. In Appel, O., Beiträge zur Kenntnis der Fusarien und der von ihnen hervorgerufenen Pflanzenkrankheiten, *Arb. K. Biol. Anst. Land u. Forstw.* 5: 157-183, illus.
- (23) SMITH, E. F.  
1899. WILT DISEASE OF COTTON, WATERMELON, AND COWPEA. (*NEOCOSMOPORA* NOV. GEN.) U. S. Dept. Agr., Div. Veg. Physiol. and Path. *Bul.* 17, 72 p., illus.
- (24) STEWART, F. C.  
1897. A BACTERIAL DISEASE OF SWEET CORN. *N. Y. State Agr. Expt. Sta. Bul.* 130, p. [423]-439, illus.
- (25) TOCHINAI, Y.  
1926. COMPARATIVE STUDIES ON THE PHYSIOLOGY OF *FUSARIUM LINI* AND *COLLETOTRICHUM LINI*. *Jour. Col. Agr., Hokkaido Imp. Univ.* 14: [171]-236, illus.
- (26) WHITE, R. P.  
1927. STUDIES ON TOMATO WILT CAUSED BY *FUSARIUM LYCOPERSICI* SACC. *Jour. Agr. Research* 34: 197-239, illus.
- (27) YOUNG, H. C., and BENNETT, C. W.  
1921. STUDIES IN PARASITISM I—TOXIC SUBSTANCES PRODUCED BY FUNGI. *Mich. Acad. Sci. Ann. Rpt.* 22: [205]-208.



# SEX RATIOS IN CUCUMBER FLOWERS AS AFFECTED BY DIFFERENT CONDITIONS OF SOIL AND LIGHT <sup>1</sup>

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## INTRODUCTION

Sex ratio in plants has been studied in some detail; sex ratio in flowers of different monoecious plants has received less attention; and no available research on the ratio of flowers of *Cucumis sativus* L. has come to the writer's attention. Because of the economic importance of greenhouse cucumbers, their behavior when grown under varying conditions of environment, as observed in commercial houses, suggested the advisability of determining the cause of some of the peculiar phenomena associated with a particular variety grown during the summer and winter months when light conditions vary widely.

Observations in commercial greenhouses where cucumbers are grown reveal the fact that cucumbers of different varieties vary in their ability to set fruit at various times during the year, some varieties setting much more than others. The cause of extreme prolificacy is undoubtedly associated with poor shape of fruit. Because of the effect which growing conditions have on the shape of fruit and the relation of these conditions to the location of pistillate flowers on the plants, an extensive study was undertaken to determine, if possible, the cause of many of the physiological and morphological phenomena which seem to be more or less closely related.

Because of the variability of genetic selections when grown during days of maximum and minimum sunlight, it was deemed advisable to determine, if possible, the effect that varying any one factor would have on the behavior of the selections, and thus establish the reliability of data collected on these selections at any one time of the year.

Cucumbers are monoecious in flowering habit, and cross-pollination is the rule. This has resulted in varieties becoming extremely heterozygous. When these varieties are selfed many different types of cucumbers may be isolated.

The fact that the Granite State variety produces a heavy "set" of pistillate flowers on the nodes of the main stem while the Belleville variety produces very few, if any, pistillate flowers on the nodes of the main stem suggests that this character at least is controlled to a certain extent by one or more genetic factors. Accepting this

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as a hypothesis, a genetic analysis of these varieties was begun, but as yet sufficient data have not been collected to show satisfactorily the relation of chromosomes to the location of pistillate flowers.

### SELF-POLLINATION STUDIES AND OBSERVATIONS

The first attempt to solve some of the difficulties of variable "set" was made by self-pollinating flowers on plants showing variations within the varieties. In the second generation, various types of both fruit and foliage characters were isolated, some of which were homozygous for certain characters, as was proved by later tests, while others again broke up in the third generation. The location of pistillate flowers was studied in detail.

A number of pure-line selections were isolated and given numbers. Among these, the selections listed below were especially interesting. These were grown during the days of rather short daylight and blossomed from January 1 to May 30, 1925. Observations on commercial varieties grown during this period show a heavy production of pistillate flowers.

21-3.—A strain bearing 2 pistillate flowers to 1 staminate flower. A very heavy set of fruit on the nodes of the main stem was followed by a large number of nubbins. Although this was not a homozygous pistillate type, it was considered a female type in later experiments. Comparatively few staminate flowers were produced.

80-4.—A normal behavior strain bearing flowers in the ratio of 1 pistillate to 6 staminate; a few pistillate flowers on the main stem.

28-4-1.—A strain bearing 1 pistillate flower to 9.5 staminate flowers. This type was referred to as a male strain. All pistillate flowers were borne on the laterals. Staminate flowers were abundant on the main stem.

C29-12.—A strain producing all pistillate flowers, many of them grown together and fasciated. Some of the nodes had 14 pistillate flowers in a cluster.

C29-10.—A normal flowering type but having a bushy habit of growth with many heavy-stemmed more or less stiff branches growing erect.

70-6.—A dwarf or short-stem strain producing only pistillate flowers, the fruit being white and short. Very few, if any, lateral branches were produced.

72-1.—A strain similar to 70-6 except that the fruit was green.

The isolation of these types grown under similar conditions indicated hereditary factors controlling flower formation and location. During the early part of the life cycle of the plants the character for flower formation seemed very flexible, while later in the life cycle when the number of staminate flowers decreased the plants became quite fixed for the pistillate condition. This was generally the case with those strains in which a heavy production of pistillate flowers occurred on the nodes of the main stem. Strains which produced only pistillate flowers late in the life cycle under maximum light conditions, under minimum light conditions tended to become stunted, and numerous fasciated pistillate flowers subtended the lateral branches and main stem.

A decided difference in vigor was noted between various selections. Most of the selections were made in second-generation plants from self-pollinated seed and were found to be homozygous for characters under observation. In height some selections were uniformly 75 cm. as compared to adjacent selections which were uniformly  $2\frac{1}{2}$  meters. Among the heterozygous types the plants ranged from 75 to 200 cm. in height. These show the normal type of growth. The dwarf types had short internodes with many lateral branches. The bushy types had many erect laterals and grew to a height of 1 meter, whereas a

vigorous normal type grew to a height of 3 meters. This difference in height was due to a difference in length of internode as the number of nodes was comparable for the various selections. These selections were made under comparable conditions of environment. How the selections would react to varying conditions of light has not been studied in all its aspects, but a few suggestions will be made later. Some of the selections did not noticeably decrease in vigor up to the sixth generation of selfing.

#### REVIEW OF LITERATURE

The review of literature in other publications is so complete that only those papers bearing on the monoecious type in plants and those relating to pertinent physiological problems have been considered in the preparation of this article.

There are many references to the general problem of sex expression in plants, and it was deemed unwise to try to harmonize the conclusions, other than those having some application to the monoecious type, with those presented later in this paper.

There is a group of investigators of sex inheritance who recognize only genetics as the determining mechanism, another group who claim that environment is the basis of sex determination, and a third, who take an intermediate position and consider the problem from several points of view.

Among those investigators who attribute the expression of sex in plants to physiological relations, which are subject to change by variations in environment, may be mentioned Burbidge (10),<sup>3</sup> Camus (11), Castle (12), Clute (13), Correns (14), Davey and Gibson (16), Fujii (18), Gardner (19), Georgeson (20), Guinier (21), Irmscher (23), Meehan (28, 29), Nagai (31), Riede (32, 33), Schaffner (34, 35, 36, 37, 38), Stout (40), and Yampolsky (41, 42).

Among those who would associate chromosomes with sex expression many be mentioned Allen (1, 2), Blackburn (4, 5), Cummings and Jenkins (15), and Janchen (24).

Blaringham (6, 7, 8), Emerson (17), and Sharp (39) show that sex is fundamentally hereditary in nature but concede that environment has its effect in the expression of the sexual state.

Some workers base their conclusions on general observations rather than on controlled experiments, so that many of those in the first group may be classified differently on further investigation. In the first group are a few who emphatically deny the possibility that heredity plays any part in the determination or expression of sex in plants. Because of the large number of species on which observations have been made and from which conclusions have been drawn without first determining whether sex characters could be established as homozygous or heterozygous states, it seems reasonable to suppose that contrary observations might result.

If sex is determined by allelomorphic genes, as has been suggested by Emerson (17), it seems especially important that the genetic constitution of material used for environmental studies be definitely established. Thus, if the factors are in a homozygous condition they would respond less readily to changes in environment than if they were in a heterozygous or weakened condition, and would be more or

<sup>3</sup> Reference is made by number (italics) to "Literature cited," p. 744.

less in harmony with Janchen's (24) suggestions that weaknesses do exist in certain individuals.

### EFFECT OF LIGHT ON FLOWER PRODUCTION

Because of the importance of the location of pistillate flowers in greenhouse cucumbers and its relation to growing greenhouse cucumbers under minimum light conditions, a study of the relation of environment was made.

Selection C29-12, a homozygous pistillate type which produced only pistillate flowers under unfavorable light conditions and a high proportion of pistillate flowers under optimum conditions, was used in studying the effect of light and nutrients on set of fruit. C29-12 is a selection of the Granite State variety. Though a selfed strain, it had sufficient vigor to be used for this work. Space did not permit the use of more than two selections in the first experiment, namely, C29-12 and 28-4-1, and these were employed in later studies.

#### EXPERIMENT 1

##### METHOD<sup>4</sup>

The seed was sown in flats in good garden soil on March 1, and when the first true leaf appeared the plants were transferred to 10-quart wooden tubs. Forty tubs were included in each of three series. Twenty of the tubs were filled with a fertile garden soil. After the plants were well started, light applications of nitrate of soda in solution were given every two weeks to keep them growing vigorously. The second lot of 20 tubs of each series was planted in a soil consisting of one-half sand and one-half garden soil by volume. No nitrogen was added to these 20 plants in each series. Series 1 received regular sunlight; series 2 received regular sunlight and also electric light from 2 a. m. to sunrise. Four ordinary 200-watt Mazda lamps were used. Series 3 was shaded from the direct sunlight by a light brown wrapping paper supported by cheese cloth. The plants were grown on strings attached to wires 6 feet above the tubs. Laterals were pinched back to three nodes. Daily records of the flowers were kept, and every pistillate flower was pollinated from another plant within the series. The plants began to flower April 26 and the experiment was discontinued June 21. Pistillate flowers which opened were counted separately from those which were formed but dried up before they opened.

##### RESULTS<sup>5</sup>

In Table 1 the summarized data show an increase of staminate flowers in the electric-light series over those in the control on fertile soil, where the plants grew vigorously to the end of the experiment at a time of the year when practically the maximum amount of sunlight was available. In the shaded series the total number of pistillate flowers per plant was reduced over 40 per cent, while the total number of staminate flowers was increased 7.7 per cent. The wide ratio of 26 staminate flowers to 1 pistillate flower is due pri-

<sup>4</sup> Unless otherwise stated, the method was the same in all of the experiments.

<sup>5</sup> In all figures and graphs the data have been corrected to 12 plants unless otherwise stated, because the average for each plant would not give sufficient data for good graphs.



marily to a decrease in pistillate flowers. In the case of the series receiving additional electric light, the increase in the ratio over the control (17 to 1 as compared to 14 to 1 for the control) is due to an increase in total number of staminate flowers. Among the plants grown in a soil low in nutrients the number of staminate flowers hardly varied in the three series, but the number of pistillate flowers was slightly smaller in the shaded series and in the series receiving electric light than in the control. The total number of both staminate and pistillate flowers was less on plants grown in unfertile soil than on those grown in fertile soil.

TABLE 1.—Average number of staminate and pistillate flowers produced on cucumber plants not exposed to direct rays of the sun; on plants exposed to normal sunlight and to electric light from 2 a. m. till sunrise; and on plants exposed only to normal sunlight; grown in fertile and unfertile soil from March 8 to June 21

FERTILE SOIL							
Treatment of plants	Average number of flowers per plant		Number of staminate flowers to one pistillate	Average number of dried pistillate flowers per plant	Average number of flowers unopened at end of experiment		Average number of pistillate flowers per plant
	Staminate	Pistillate			Staminate	Pistillate	
Received normal sunlight (control).....	174.5	12.3	14.2	54.0	12.4	18.9	85.2
Received normal sunlight and also electric light from 1 a. m. till sunrise.....	210.5	12.4	17.0	46.3	12.8	32.9	91.6
Shaded; no exposure to direct rays of sun.....	188.0	7.1	26.5	29.5	35.3	22.5	59.1
UNFERTILE SOIL							
Normal sunlight (control).....	136.5	9.4	14.5	28.2	2.3	8.9	46.5
Received normal sunlight and also electric light from 2 a. m. till sunrise.....	136.5	7.8	17.5	25.5	1.8	3.7	37.0
Shaded; no exposure to direct rays of sun.....	138.5	8.8	15.7	20.9	4.3	2.6	32.3

In Figure 1, C29-12 and 28-4-1, two self-pollinated selections, and C100, a hybrid (Belleville variety), are compared for flowers. These plants were grown side by side in a greenhouse bed from February 1 to April 30. The ratio of 2 staminate flowers to 1 pistillate flower for C29-12 is considerably lower than the ratio secured from the experiment (14.2 staminate : 1 pistillate) during a period of days having more intense sunlight for a longer period each day. Comparing the average number of flowers for each plant, the narrow ratio in Figure 1 (2 staminate : 1 pistillate) is due to a decided decrease in staminate flowers and an increase in pistillate flowers. Thus a difference in amount and intensity of light seems to have a decided effect on the production of flowers. A difference of 30 calendar days has a marked effect on the plants because of the difference in the amount of light which they receive at a time when they are forming flower primordia. In Figure 2 a comparison of the control with the electric-light series is shown graphically for the duration of the experiment. Electric light increased the production of staminate flowers (electric light 17 staminate : 1 pistillate; control 14.2 stami-

nate : 1 pistillate) during the latter part of the life cycle of the plants. The effect during the first part of the cycle was not so noticeable. The difference noted suggested growing plants from the same lot of seed during days of reduced sunlight.

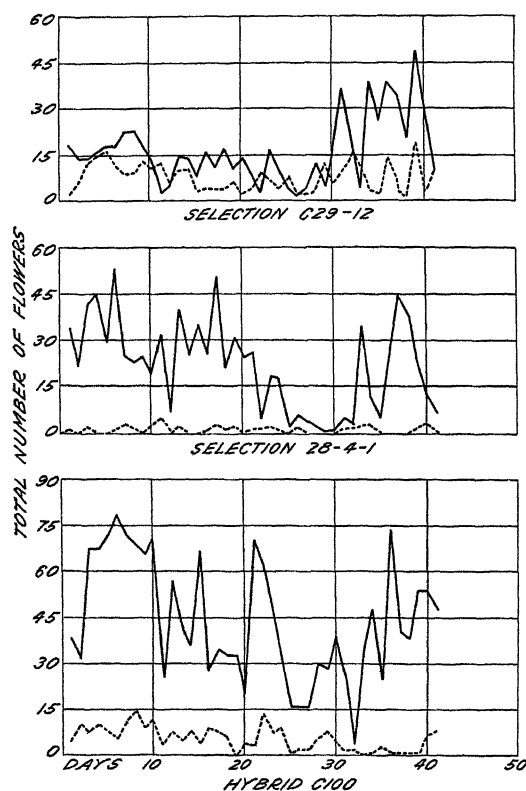


FIG. 1—Comparison of two selfed selections and a hybrid for staminate and pistillate flowers, pistillate flowers are shown by dotted lines; staminate flowers by solid lines. Data were taken February 1 to March 15

9 a. m. In each of the three series half of the plants were grown in poor soil, and the other half in very fertile soil.

## EXPERIMENT 2

### METHOD

As in experiment 1, the plants in experiment 2 were grown in three series. 1. Control plants. 2. Plants receiving regular sunlight and electric light. The lights were turned on at 2 a. m. and allowed to remain on until sunrise. On cloudy days they were lit until 6 p. m. The lights were placed 5 feet above the plants, and as the plants grew higher they were gradually raised. The intensity of the light at the top of the plants was 100 foot-candles, which reduced the amount of steam necessary to keep the compartment at 65 F. during the night. No difference in the air temperature was noticeable between this series and the control. 3. Plants placed on a truck were run into a dark chamber at 3 p. m. and taken out at

## RESULTS

In Table 2 a summary of the data obtained in experiment 2 is given. The ratios here shown for control plants agree very favorably with the ratio for C29-12 in Figure 1. The total number of staminate flowers for each plant is considerably lower than for the same selection as shown in Table 1, where the data are for plants grown under maximum light conditions. The number of pistillate flowers was very much the same. Thus a reduction in the intensity and duration of the daylight decreased the number of staminate flowers.

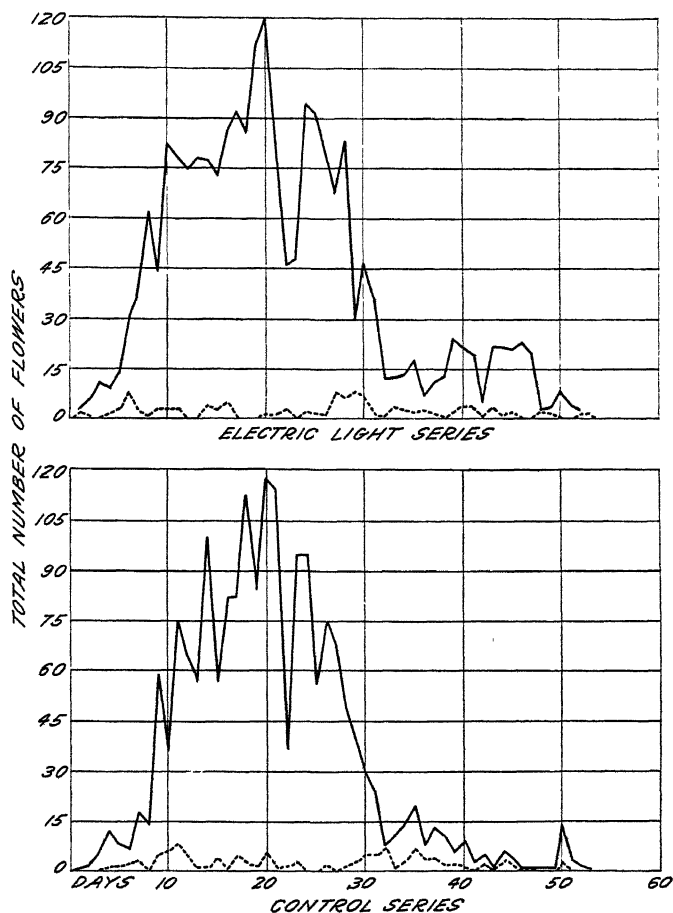


FIG. 2.—Behavior of a strong pistillate flower selection, C29-12, for staminate and pistillate flower production with and without additional electric light, when grown during days of relatively long sunlight. Pistillate flowers are shown by dotted lines, staminate flowers by solid lines

TABLE 2.—Average number of staminate and pistillate flowers produced on selection C29-12 when plants were shaded from 3 p. m. to 9 a. m.; when they received normal sunlight and also electric light from 2 a. m. to sunrise and on cloudy days; and when they received sunlight only; grown in fertile and unfertile soil from December 1 to March 1

Treatment of plants	Fertile soil			Unfertile soil		
	Average number of flowers per plant			Average number of flowers per plant		
	Staminate	Pistillate	Number of staminate flowers to one pistillate	Staminate	Pistillate	Number of staminate flowers to one pistillate
Sunlight only (control).....	16.4	12.9	1.3	14.0	9.3	1.5
Normal sunlight; electric light from 2 a. m. to sunrise and on cloudy days.....	37.5	13.0	2.9	37.0	10.6	3.5
Shaded from 3 p. m. to 9 a. m.....	4.2	3.7	1.1	10.2	4.0	2.6

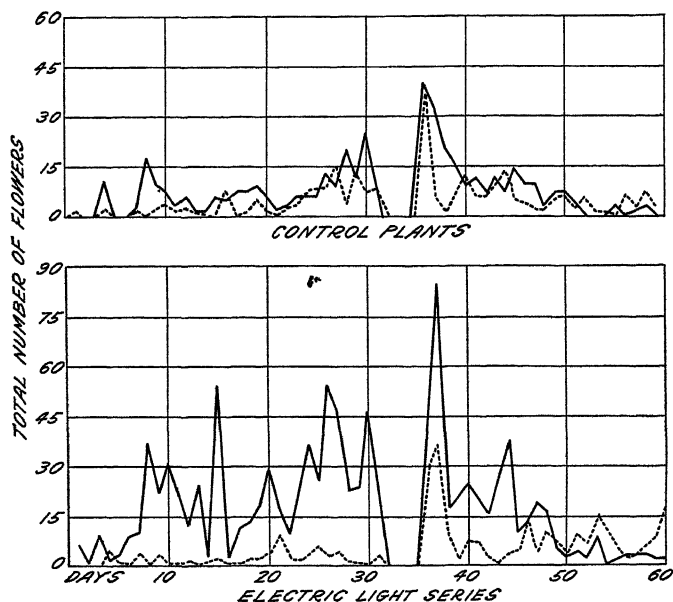


FIG. 3.—Effect of electric light on the production of flowers on plants grown during days of minimum sunlight. The gap after the thirty-second day was caused by naphthalene fumigation which prevented the flowers from opening. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

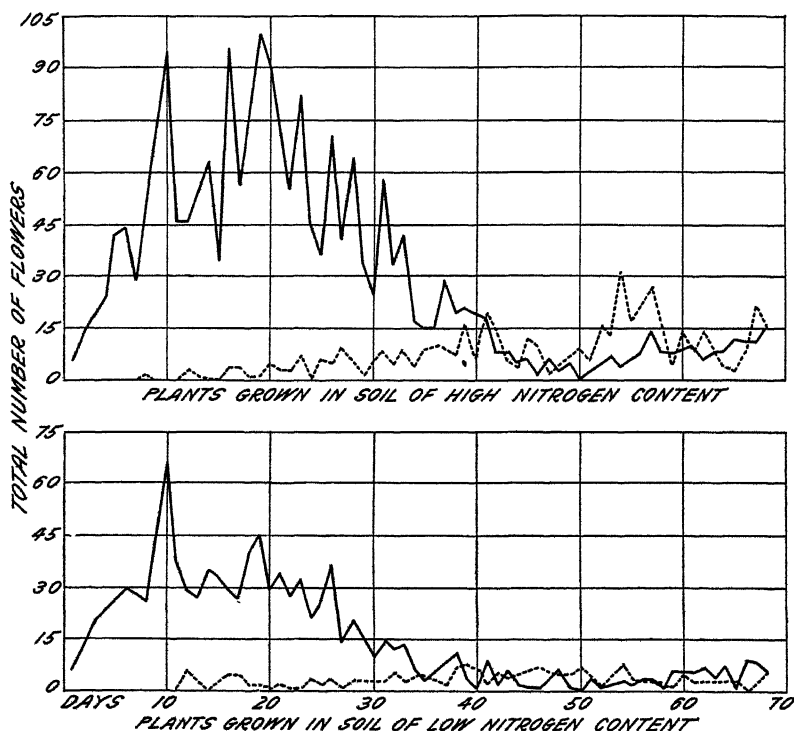


FIG. 4.—Effect on the production of pistillate and staminate flowers of growing plants in soil of high and low nitrogen content during days of relatively long sunlight. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers

Comparing the electric-light series with the control series, a material increase is noted in the production of staminate flowers in the electric light series, as is shown graphically in Figure 3. Shading the plants reduced both staminate and pistillate flowers. (Table 2.) The fertility of the soil increased the number of pistillate flowers and slightly reduced the ratio. The greatest difference is shown in the shaded plants, where more staminate flowers were produced.

#### EFFECT OF FERTILITY OF SOIL ON FLOWER PRODUCTION

In these studies a fertile soil is discussed in terms of nitrogen even though the specific effect of nitrogen in every case is not known.

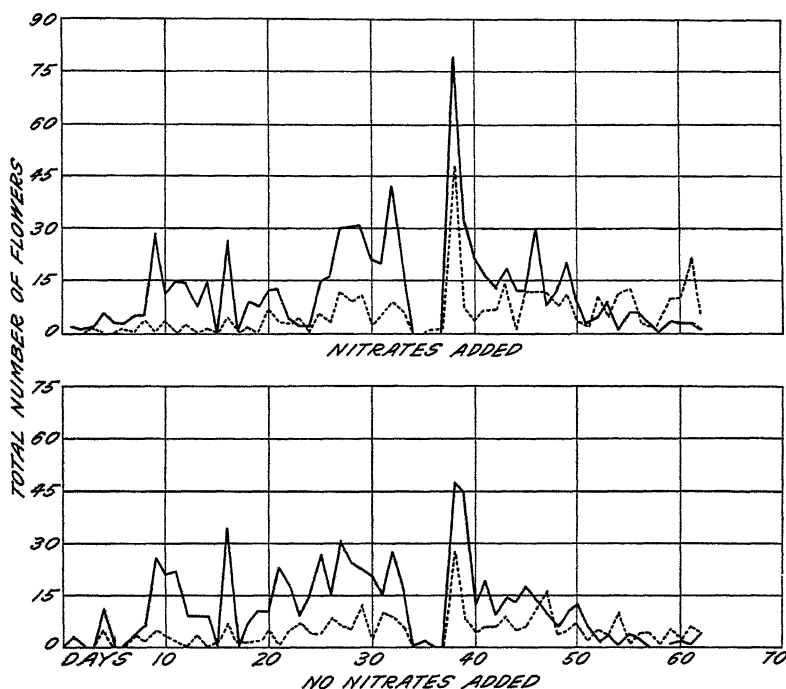


FIG. 5.—Effect on the production of pistillate and staminate flowers of growing plants in soil of high and low nitrogen content during days of minimum sunlight. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers

The common observation is that if cucumber plants become yellow and nitrogen is used in one of five different combinations  $\text{KNO}_3$ ,  $\text{NaNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea,  $(\text{NH}_4)_3\text{PO}_4$ , the effect is to cause the plant to become quite green, the degree of color depending on the amount added. Too much at one time causes burning at the tips of the leaves.

In Figures 4 and 5 the effect of growing plants in a soil of high nitrogen content is shown. During the days of maximum sunlight there was a total increase in staminate flowers on the plants grown in high nitrogen soil of 45.5 per cent, and an increase in pistillate flowers of 55.08 per cent. During the days of minimum sunlight there was an increase in staminate flowers of only 3.14 per cent, but in pistillate flowers there was an increase of 20.67 per cent. A com-

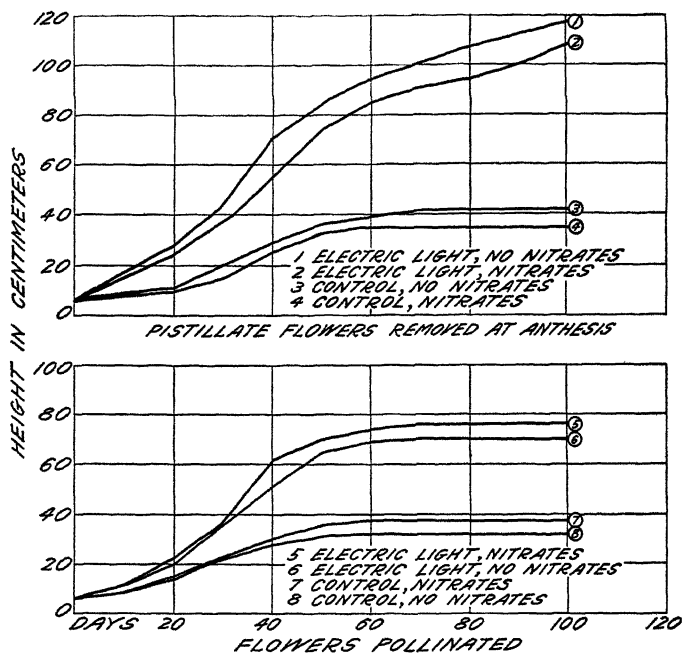


FIG. 6.—Growth rate of plants receiving normal sunlight and electric light or normal sunlight only (control), grown in soil with and without nitrates during days of relatively short sunlight. The upper set of curves present data for plants from which the pistillate flowers were removed at anthesis; the lower set present data for plants on which all pistillate flowers were pollinated.

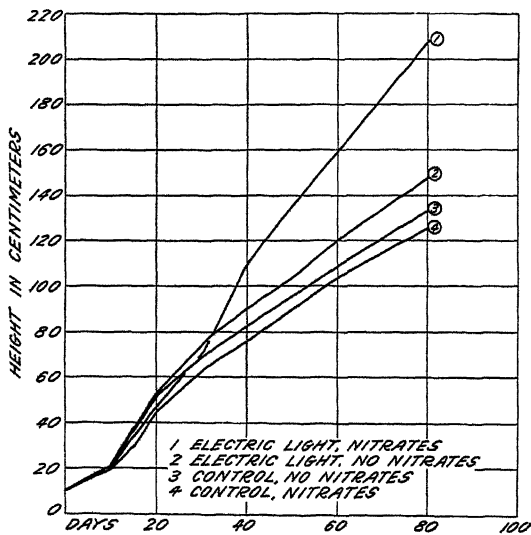


FIG. 7.—Growth rate of plants receiving normal sunlight and electric light or normal sunlight only (control), grown in soil with and without nitrates during days of relatively long sunlight.

parison of Figures 4 and 5 indicates that the utilization of nitrogen is dependent on the light increment or sugar content of the plant.

In Figures 6 and 7 growth curves for these plants are shown. Figure 6 shows the effect of light and nitrogen during the days of relatively short sunlight. The wide difference in final height of plants is indicative of the important effect which a small increase in light exerts during the short days. The control plants stopped growing when 32-37 cm. high and produced a cluster of pistillate flowers at the tip with no lengthening of the internodes.

Plants grown under days of relatively long sunlight showed a much more rapid rate of growth, and only where nitrogen was added did

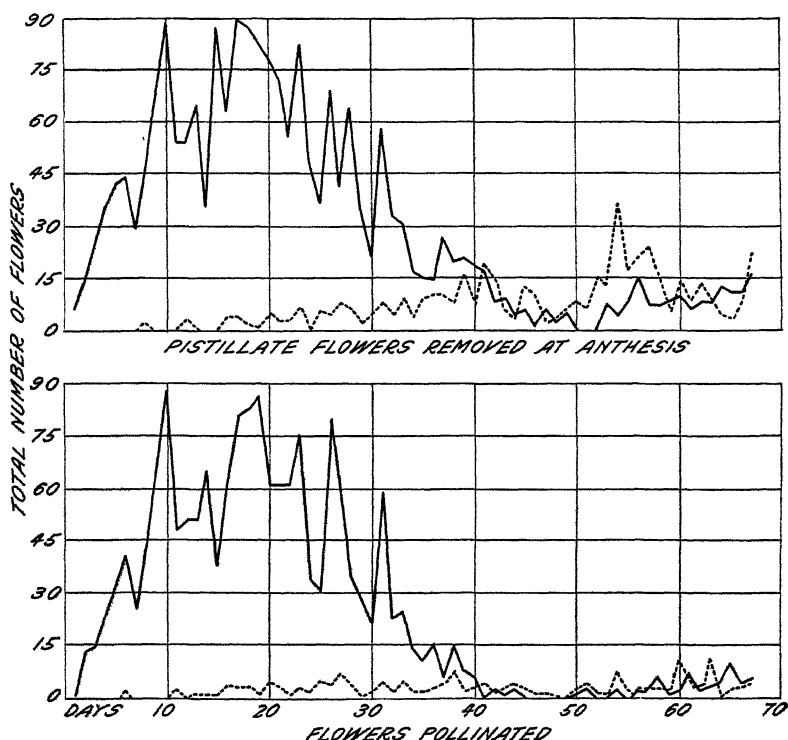


FIG. 8.—Effect of removing all pistillate flowers at anthesis on the later production of pistillate and staminate flowers. These plants were grown during days of relatively long sunlight. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

the additional electric light cause a significant increase in rate of growth. These growth curves are the result of data taken on the main stems of the plants. It is interesting to note that even though the plants grown in soil of high and low nitrogen content differed widely in appearance, the rate of growth of their main stems was similar.

#### EFFECT OF DEVELOPING FRUITS ON THE RATIO OF STAMINATE TO PISTILLATE FLOWERS

The production of new flowers on plants soon after a mature fruit was picked suggested an experiment in which the pistillate flowers on some plants were removed at anthesis, while other plants were per-

mitted to mature any pistillate flowers which opened. No definite number of fruits was left on the plants because a plant regulates the number that it will carry. There are always more pistillate flowers produced than the plant can successfully mature. If the pistillate flowers are all pollinated and become fertilized, the first ones will start to grow and establish an inhibitory effect on those pollinated later. The extent to which this inhibitory effect is manifested differs in different plants. One plant, for example, may develop only one fruit at a time, while another, very similar in manner of growth, may develop five or six. Flowers that are pollinated simultaneously usually grow equally well.

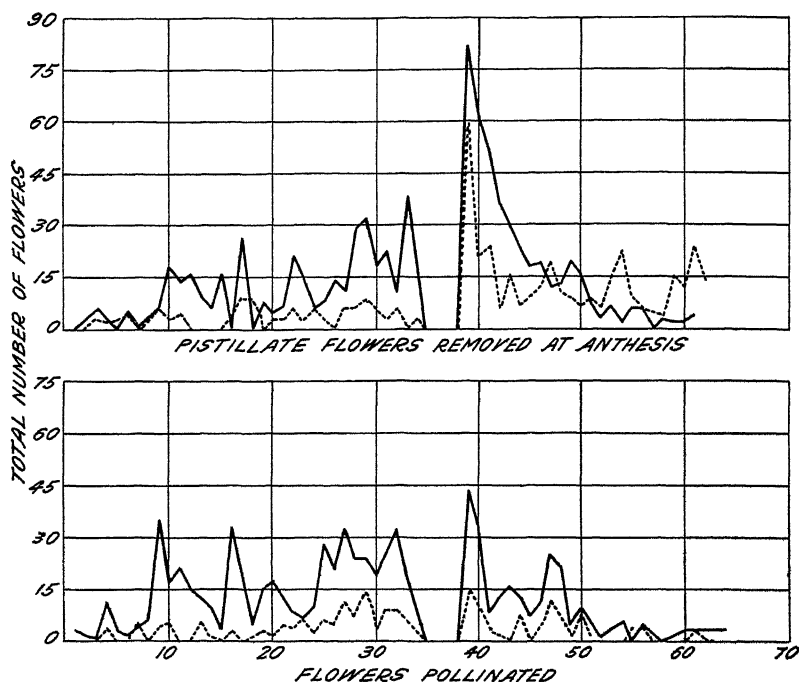


FIG. 9.—Effect of removing all pistillate flowers at anthesis on the later production of pistillate and staminate flowers. These plants were grown during days of minimum sunlight. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

Pistillate flowers which have been fertilized but inhibited from growing because of one or more developing fruits may remain inactive for as long as 30 days or until the growing fruits have been removed, and may then resume growth and sometimes develop into very well-shaped fruits. Usually, however, early inhibition results in poorly shaped fruit because of the maturity of some of the tissue, which turns yellow soon after the cucumber begins to grow. It is not uncommon to find the growth cycle of a fruit interrupted two or three times before it reaches maturity.

In Table 3 data are given for plants with and without fruit grown from April to June, and in Figure 8 the results are shown graphically for the same plants. The removal of the flowers at anthesis in every case increased the production of pistillate flowers and reduced the ratio of staminate to pistillate flowers at least 40 per cent (control 7.9



staminate : 1 pistillate, pistillate flowers removed 3.9 staminate : 1 pistillate). In Figure 9 the effect of removing pistillate flowers on the subsequent production of flowers is shown for plants grown during days of minimum sunlight. The differences shown are similar to those in Figure 8. The pistillate flowers increased 56 per cent while the staminate flowers increased only 8 per cent. The plants were 100 per cent pistillate at the end of the experiments.

TABLE 3.—Average number of staminate and pistillate flowers produced on plants of selection C29-12 when fruits were allowed to remain on vines and when they were removed; plants grown in fertile and unfertile soil from April to June

Treatment of plants	Fertile soil			Unfertile soil		
	Average number of staminate flowers	Average number of pistillate flowers	Number of staminate flowers to one pistillate	Average number of staminate flowers	Average number of pistillate flowers	Number of staminate flowers to one pistillate
Shaded:						
Fruit left on .....	113.0	8.3	13.6	96	5.5	17.4
Fruit removed .....	160.5	18.0	8.9	103	8.9	11.6
Sunlight and electric light.						
Fruit left on .....	79.8	7.8	10.2	80	6.5	12.3
Fruit removed .....	94.0	25.3	3.7	69.4	11.6	6
Normal sunlight (control):						
Fruit left on .....	99.1	10.8	9.2	68.7	5.3	13
Fruit removed .....	107.0	25.8	4.1	72.8	14.0	5.2

\* All pistillate flowers were pollinated on plants carrying fruit.

In Figure 8 the cyclic production of flowers is shown. The general tendency of the plant under optimum light conditions is to produce a large number of staminate flowers early in the flowering cycle, and as the pistillate flowers are fertilized and the fruit begins to grow, the production of staminate flowers practically ceases. As the fruits are removed when mature (not ripe), flowering begins again, but few staminate flowers are produced, with the result that the later pistillate flowers are improperly fertilized and the fruit does not develop well.

Before this fact was known, considerable difficulty was experienced in securing hand-pollinated or controlled fertilized fruit for genetic studies. During days of long sunlight, unless controlled pollinations could be made on the first pistillate flowers, the practice was to remove all pollinated fruit before making any controlled hand pollinations. During days of short sunlight the removal of all fertilized fruits on such selections as C29-12 resulted in the production of practically all pistillate flowers on the new growth, so that self-pollinations were impossible. These plants must be started in a rather unfertile soil and the first pistillate flowers utilized in order to get self-fertilized seed. The use of electric lights will facilitate matters somewhat because more staminate flowers will be produced.

#### EFFECT OF ENVIRONMENT ON THE APPEARANCE OF THE PLANTS

When a cucumber plant is grown in a pot under favorable light conditions it soon uses its available nitrogen and begins to turn yellow. The leaves are small and an abundance of very small staminate flowers make their appearance at the nodes of the main

stem, and if any laterals have been formed they will have clusters of staminate buds which may or may not open. The laterals remain short, even though four or five nodes have been formed. The internodes are very small in diameter and quite yellow, and the clusters of numerous staminate buds are conspicuous because of their very short pedicels. The plant produces only sufficient sustaining tissue to support the flower buds. Whether these buds will open depends on the amount of nitrogen available to the plant. In the last stages of nitrogen starvation the nodes at the tip of the main stem remain active while the leaves on the lower nodes turn a greenish yellow and finally become quite white, after which they soon become desiccated. Finally the plant reaches the condition shown by the two in Figure 10, A, *a* and *b*. An occasional pistillate flower may be produced at the tip. When the plant can not get its nitrogen from the soil it seems to draw on any surplus in the larger leaves and to transport it to the growing tip, where pistillate flowers may be formed even though the new leaves are very small.

If some form of soluble nitrogen such as urea is added to the soil, a noticeable change in the plants is visible in 24 hours, and in 12 days this results in a condition such as that shown by the two plants in Figure 10, A, *c* and *d*. The older leaves do not become green and resume growth, but gradually turn hard and stiff, while the new leaves assume the appearance of those of a plant receiving sufficient nutrients for luxuriant growth. The new stem becomes large and rugged in appearance while the old stem remains small and yellow. Even though the stem at the base remains small no ill effects from lack of water in the new growth are shown. Figure 10, A, *d*, shows a cucumber developing normally from a flower which has been pollinated previous to the application of urea.

Under reduced light conditions the effect of nitrogen is considerably different on plants producing abundant pistillate flowers on the main stem. In place of the continuation of normal growth, no appreciable extension of the main stem results, but pistillate flowers are thrown out from all nodes, even where dried leaves have been removed. In Figure 10, B, sections of the main stem from a green and a white-fruited plant of the heavy main stem "set" type are shown. The leaves are small, rather long, with very short petioles, which gives them a dwarfed appearance. Under optimum light conditions the fruits on the plant shown in Figure 10, B, *b*, would have prevented the further development of pistillate flowers. Under reduced light conditions the presence of developing fruits has little effect, and large numbers of pistillate flowers are formed even though they seem to be sterile. The tip of the stem becomes a mass of pistillate flower buds and the new internodes become shortened. Theoretically, it would seem as though all fruit tissue and no stem tissue would be the final result. Even though the differentiation of parts take place, development of those parts is handicapped by a lack of some-

#### EXPLANATORY LEGEND FOR FIG. 10

A.—*a* and *b*, plants which show nitrogen starvation; *c* and *d*, plants which show the response to nitrogen 12 days after the application of urea.

B.—Sections of the main stems of, *a*, a white and, *b*, a green-fruited cucumber plant, showing the effect of reduced light on the growth of leaves and increase in pistillate flower production.

C.—Parthenocarpic fruits developed under reduced light on a pistillate selection which will not develop parthenocarpic fruits under optimum light conditions. The small fruit shows partially developed ovules. Sometimes the seed coat is formed as in a fertilized fruit, but it is always empty.

D.—Fruits similar to those shown in C; *a*, seed cavities.

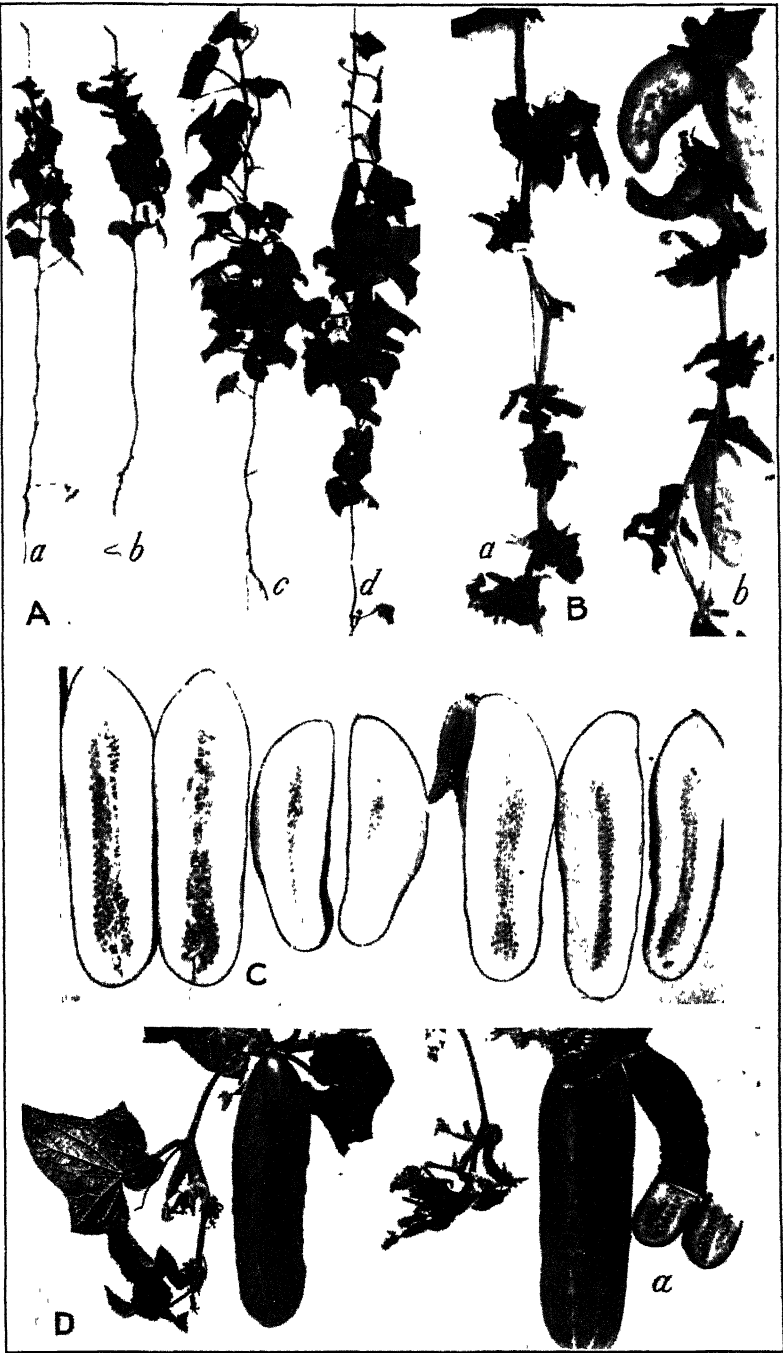


FIG 10.—*Cucumis sativus*  
(For explanatory legend see p. 734)

thing, undoubtedly carbohydrates, for either cell-wall formation or elaboration of proteins. Under these conditions, of which the plants in Figure 10, B, are the result, a false parthenocarpic condition is brought about. Some seeds developed in the three large fruits as a result of an earlier light effect, but the smaller fruits were not pollinated. When the pistillate flowers are pollinated, fertilization does not seem to be accomplished even though good viable pollen is used. Apparently insufficient sugars are available to feed the pollen tube in its growth into the stigma. Brink (9) has shown that a rather high concentration of sugar is necessary to bring about germination of the pollen grains. It is not uncommon to find well-shaped fruit being produced without fertilization on such vines under unfavorable light conditions, as is shown in Figure 10, C and D. Under high nitrogen and optimum light conditions, plants from selections like C29-12 produce only one pistillate flower at a node, and unless these are pollinated they immediately dry up after anthesis.

In Figure 11, A, some forms of fasciation found on "pistillate" plants under reduced light conditions are shown. All stages from fasciation of the petioles of staminate flowers to so-called double ovaries may be found, though it must be understood that not all pistillate plants grown under reduced light produce fasciation. The short petioles are characteristic.

To say that this fasciated condition is due entirely to light or entirely to nitrogen would seem unreasonable. Under reduced light conditions a given quantity of nitrogen will keep the plant green for a longer period than under optimum light conditions. Even though a certain amount of nitrogen is necessary for the growth of the plant, yet the available sunlight, quantitatively and qualitatively speaking, seems to be the controlling factor in the abnormal prolificacy of some strains of cucumbers and the false parthenocarpic production of fruits.

The presence of a maturing fruit may influence the reaction of the plant to light. As has already been shown, the removal of the pistillate flowers at anthesis results in the subsequent production of a greater number of pistillate flowers. In Figure 11, B, is shown a plant from selection 90-1-3 which had four pistillate flowers pollinated on the same day and all four of the resultant fruits were permitted to ripen. The plant produced few laterals and the main stem had grown 230 cm. when the picture was taken. A plant (fig. 11, C) from the same selection had no flowers pollinated and grew 387 cm. in the same time. More laterals were produced and the tip of the stem showed a luxuriant, vigorous growth as compared to a slender unproductive growth in the other plant. Both were grown under optimum light conditions. Under greatly reduced light the effect of fruit on the growth of this type of plant is not so pronounced because the available light apparently is the controlling factor for stem elongation.

For the prolific "set" type, the plant shown in Figure 12, A, is a good illustration. For this type the time of pollination is important. If a flower has been fertilized several weeks before the plant is subjected to reduced light, the ripening fruit will have some inhibitory effect on the production of pistillate flowers, and not until the fruit is removed or dropped will the final stage shown in Figure 10, B, result. In this case the amount of nitrogen available at certain stages may

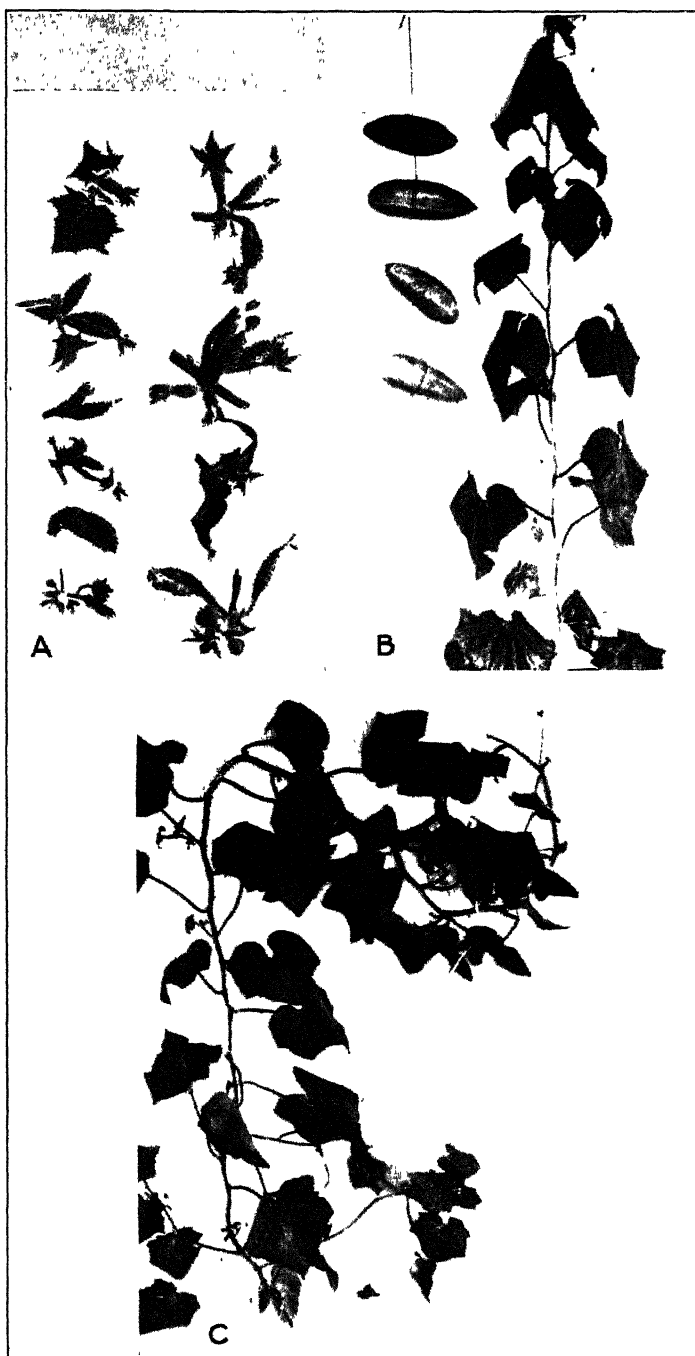


FIG. 11 —A, clusters of pistillate flowers characteristic of the C29-12 type under minimum light conditions; B, plants showing influence of developing fruit on growth; C, a plant from the same selection as B, but having no flowers pollinated



FIG. 12.—A.—C29-12, a pistillate type. The absence of staminate flowers at the nodes is characteristic of plants of this type when grown under partially reduced light. B.—Plant showing effect of heavy nitrogen applications when grown under optimum light conditions. C.—28-4-1, a staminate strain. An occasional pistillate flower is visible on the laterals. D.—A pistillate flower of unusual size at anthesis. Occasionally found on plants during days of reduced sunlight

bring about the extreme fruiting condition. These results are more or less in agreement with Murneek's (30) results with tomatoes.

There should be mentioned a few specific effects of nitrogen under a given light condition. In Figure 12, B, is shown a plant grown with optimum nitrogen under favorable light conditions. Parts of this plant are shown in detail in Figure 13, C. This plant produced pistillate flowers at the nodes on the main stem. In Figure 12, C, the type is shown with pistillate flowers on the laterals only. The luxuriant green growth is very conspicuous.

The presence of nitrogen in the soil has a pronounced effect on the size of the flowers. Figure 12, D, shows a flower at anthesis and

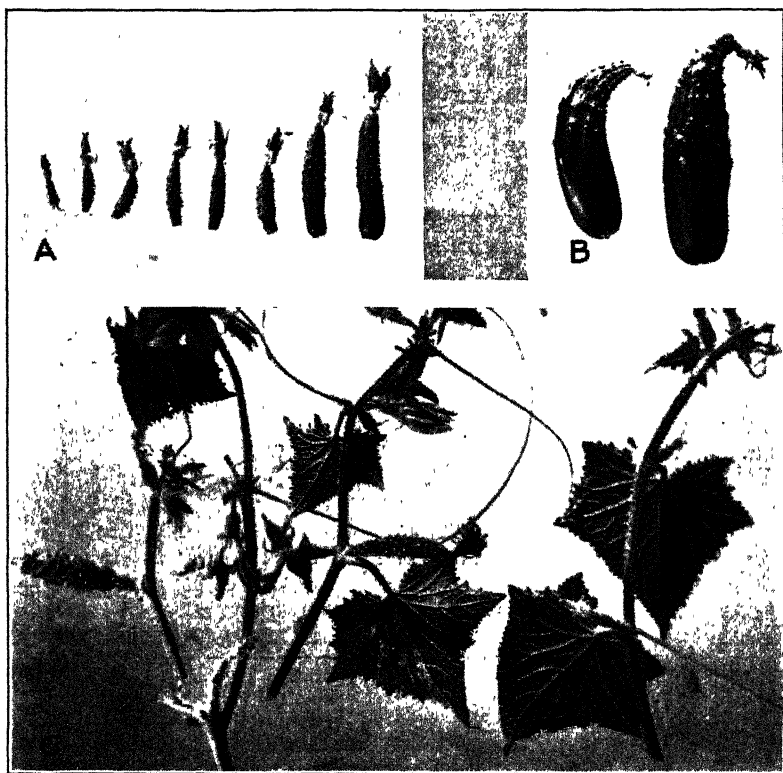


FIG. 13.—A.—Ovaries of flowers 10 hours before anthesis, showing variation in size when plants are grown under reduced light and different conditions of nutrition. B.—Unusually large ovaries of flowers 10 hours before anthesis found on plants grown under reduced light; rather a common occurrence. C.—A closer picture of the young leaves from laterals of plant shown in Figure 12, B

Figure 13, A, shows pistillate flowers 12 hours before anthesis. The difference in size can be attributed to increased amounts of nitrogen. The effect of vigor is shown by some measurements of pistillate flowers taken after all developing fruits had been removed from a vine producing a 30-cm. mature fruit. The ovaries on the first pistillate flowers averaged 5.8 cm. in length at anthesis. Four days later the average at anthesis was 4.8 cm., and in 14 days the average at anthesis was 3 cm.; at which time the first fertilized fruits had attained a length of 24 cm. Thereafter all flowers that did not dry up before anthesis averaged 3 cm. A large number were produced after the maturing

fruits had reached their maximum size but rarely did one reach anthesis, because they turned yellow when the ovary was from 1 to 3 cm. long. Apparently the amount of nitrogen available when all pollinated fruits were removed previous to taking measurements of length of ovaries was in a large measure responsible for the abundant formation of pistillate flower primordia, a condition which prevailed as long as the laterals continued to grow. As the developing fertilized fruits required more nutrients, less was available for the extension of laterals, and the elaboration of pistillate flowers decreased, until finally a large number of them turned yellow before anthesis. Under decreased nitrogen conditions fewer pistillate flower primordia are produced. Although sufficient light is necessary yet the abundant nitrogen above the optimum results in the production of more pistillate flowers, the ovaries of which are considerably larger than normal. In Figure 13, B, ovaries are shown of a type which is common on heavy-set plants during days of minimum sunlight provided the soil is quite fertile. The larger ovary in Figure 12, D, was 7 cm. long just before anthesis.

#### EFFECT OF ENVIRONMENT ON THE RATIO OF STAMINATE TO PISTILLATE FLOWERS

In selection C29-12, a prolific pistillate flower type, the ratio of staminate to pistillate flowers was changed from 10.8:1 during days of comparatively long sunlight to 1.8:1 during days of comparatively short sunlight. Toward the end of the experiment C29-12 was 100 per cent pistillate. This condition is characteristic of plants of this type which have a pistillate flower at practically every node and a reduced number of staminate flowers.

In selection 28-4-1, a selection producing very few pistillate flowers and those only on the laterals, and producing staminate flowers very prolifically at all the nodes, it is possible to change the ratio of staminate to pistillate flowers, but here the type is at the opposite end of the scale. Under optimum light conditions this selection gave 64.0 staminate flowers to 1.0 pistillate flower, as shown in Figure 14, with some plants producing no pistillate flowers, while under reduced light conditions a ratio of 25.5 staminate flowers to 1 pistillate flower is the result with all plants producing some pistillate flowers.

The significance of these facts is that under optimum light conditions C29-12 can be shifted from a normal condition to 100 per cent pistillate, while 28-4-1 can be shifted from practically 100 per cent staminate to the normal type. C29-12 could be shifted to the pistillate type but not to the staminate type, while 28-4-1 could be shifted to the staminate type but not to the pistillate type, all by changes of the environment. Thus, these types are flexible within certain limits, and these limits are undoubtedly controlled by the genetic mechanism of the plant. In C29-12 under optimum light conditions numerous staminate flowers are produced at each node with the pistillate flower. As the light intensity and duration are decreased fewer staminate flowers are produced until in December, under North Temperate Zone conditions, only pistillate flowers occur. The difference here noted in behavior of strains agrees with the results of Correns (14) on *Satureia hortensis* (summer savory), in which different strains gave different ratios of perfect to pistillate flowers. In 28-4-1 there is a decrease in staminate flowers, while the



pistillate flowers are only slightly increased. In the case of a commercial variety, the degree of shifting of the ratio would depend on the genetic potentialities of the variety. This fact is very well demonstrated by the Granite State and Belleville varieties. Under reduced light conditions the Granite State has a very narrow sex ratio. Under optimum light conditions the sex ratio is approximately 6 staminate flowers to 1 pistillate flower. In the Belleville strain under reduced light conditions, the sex ratio is normal and becomes wide under optimum light conditions. It is possible to combine certain isolated strains which will produce sex ratios within certain desired limits.

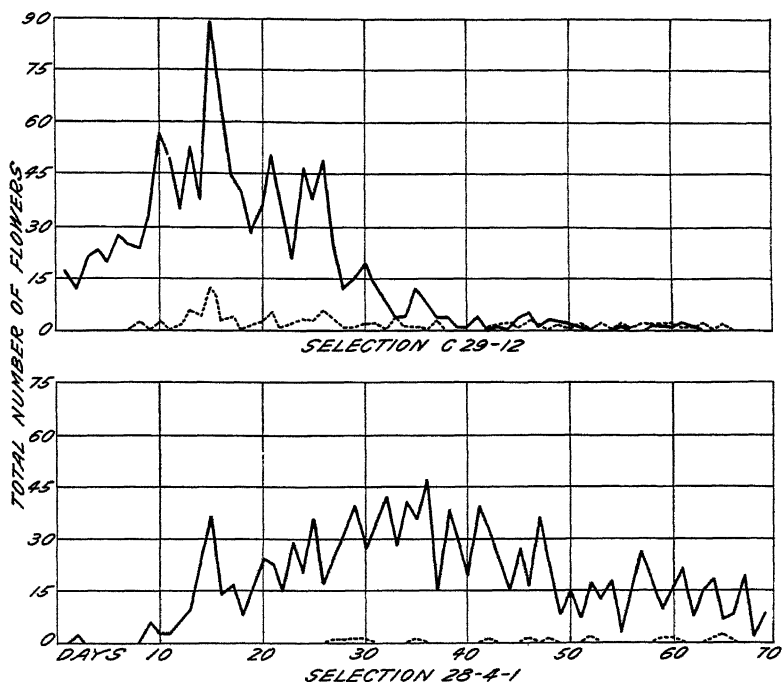


FIG 14.—Comparison of selections C29-12 and 28-4-1 for distribution of pistillate and staminate flowers when grown under comparable conditions from September to November. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers. Compare with plants in Figure 1, grown during days of maximum sunlight

Cytological data of a preliminary nature using Bellings acetocarmine method have not disclosed any departures from the normal seven haploid chromosomes established by Kozhukhov (25) and later by Heimlich (22).

Preliminary data on the genetics of greenhouse cucumbers indicate that pistillate and staminate flowers result from factor genes on the chromosomes rather than from any difference in the number or shape of the chromosomes. These conclusions are in agreement with Emerson's (17) hypothesis that sex in plants is determined by genetic factors just as any other characters are determined by genes on the chromosomes.

Data on staminate and pistillate flowers of an  $F_2$  population show plants having a large number of staminate flowers and very few

pistillate flowers with all intergrading variations to the type that has abundant pistillate flowers and very few staminate flowers under a given set of environmental conditions. From the data the conclusion may be drawn that on any given selection, optimum light conditions (abundant carbohydrates) favor the abundant production of staminate flowers even where nitrogen in quantity considerably above the optimum is applied to the soil. It may also be concluded that reduced light conditions favor the production of pistillate flowers with a decided decrease in staminate flowers regardless of an appreciable fluctuation of the nitrogen content of the soil. This suggests a correlation between flower formation and the amount of available carbohydrates and nitrogen which Kraus and Kraybill (26) call the nitrogen-carbon ratio. Whether it is due to the ratio of carbon and nitrogen or carbon and some other soil element must be determined by a careful chemical analysis. The reactions of the plants when nitrogen is added suggest that flower production within the limits of gene flexibility is correlated with some carbon-nitrogen relationship which probably is not a fixed ratio for all plants.

#### DISCUSSION

Manoilov (27) combined certain chemicals which reacted differently to staminate and pistillate tissue. Tests were made with these reagents, and these tests showed plants which were almost 100 per cent pistillate, as selection C29-12, to have a different reaction from plants which were decidedly staminate, as 28-4-1. Leaves of plants which were segregating in the  $F_2$  generation showed decided differences when subjected to this test, indicating a difference in the metabolic constituents of the plants. This difference was noticeable whether the day was dull and cloudy or bright and sunny. Tests made on young and mature leaves from the same plant showed no detectable difference. These observations are in agreement with certain microchemical tests which showed that pistillate plants were deficient in sugar but high in nitrogenous substances. Staminate plants showed more sugar in the leaves but were also rather high in nitrogenous substances. Alsterberg and Håkansson (3) have criticized Manoilov's test rather severely because positive results were not secured on all plants. Undoubtedly the test is influenced by the homo- or hetero-zygosity of the sex condition, the former probably giving a better test than the latter. It was found in tests on cucumbers that extreme care is necessary in applying the test.

The general conclusion from the data lends support to the hypothesis that sex is determined by some genetic-factor mechanism which controls the metabolism in the plants rather than the actual character of sex expression, and that the ratio between the two types of flowers is the result of this nutrient condition. Each plant may have a definite nutrient ratio which is thrown one way or the other by light and by soil nutrients. Kraus and Kraybill (26) suggest a nitrogen-carbon ratio as controlling the reproductive stage in tomatoes. If this ratio is in a normal pistillate plant containing the recessive factor for flower location as in selection C29-12, a decrease in light will throw it toward the extreme pistillate type. Where the ratio is accompanied by the dominant allelomorph producing pistillate flowers only on the laterals, as in 28-4-1, a decrease in light will likewise cause the plant to produce more pistillate flowers, but here,

because of the inhibition of pistillate flowers on the nodes of the main stem, the ratio remains rather wide.

The presence or absence of fruit may influence this ratio somewhat, but in experiments in which all the pistillate flowers were removed the plants immediately proceeded to repeat the previous cycle, in keeping with their genetic potentialities under a given environment. The effect of the presence of fruit and other conditions of environment may be seen, but their influence is confined within certain limits established by the genetic factor mechanism of the plant.

Just how this relationship interacts is a question that a very careful chemical study may answer. The retarded growth of the C29-12 type is not due to the heavy production of pistillate flowers, for there are types which are free growing and produce pistillate flowers on practically all the nodes. Selection 28-4-1 is a free-growing type without the pistillate flowers on the nodes of the main stem. From breeding data it would seem as though some plants are endowed with certain potentialities which decide their ratio of carbohydrates to soil nutrients and that the production of pistillate and staminate flowers is the result of this ratio. Selection C29-12 would then produce all pistillate flowers when the ratio stood, let us say, 1 carbon unit to 5 soil-nutrient units, while in 28-4-1 the ratio would have to be 1 carbon unit to 10 soil-nutrient units to produce only pistillate flowers; a condition which has never been observed in these experiments. Under a 1 : 10 ratio, selection 28-4-1 might be induced to produce pistillate flowers on the nodes of the main stem. Even were this condition attained, it would not invalidate the genetic hypothesis discussed above, for there would still be the previously noted difference between the two selections, which can only be explained by some basic determining mechanism. The inhibited type of growth, as in C29-12, seems to possess a tendency to utilize the sugars for protein metabolism, while the 28-4-1 type has a tendency to accumulate sugars. It is possible that the genetic factor influences the quality of the sugar in the different types of plants, so that the reduction of nitrates might result in differently constituted proteins which would have their respective resultants in the appearance of the plants and their behavior for flower formation.

The general conclusion would support the hypothesis suggested by Emerson (17) that sex is determined by some factor or gene on one or more pairs of chromosomes, which is more or less flexible to allow for the effect of environmental changes. It further suggests that the sex ratio is the result of a chemical balance in the plant which probably is the medium through which the factors show their influence. Whether the initial cell produces a staminate or a pistillate flower probably depends on the inter- and intra-cell nutrients. In the 28-4-1 type where a factor inhibits the production of pistillate flowers at certain nodes, these cell nutrients will result in either staminate flowers or stem and leaf tissue, depending on environmental conditions. Where the inhibitory factor is lacking, pistillate flowers will be formed accompanied by staminate flowers or other pistillate flowers, depending on environmental conditions. If conditions are not conducive to the formation of a single pistillate flower the node may remain barren.

Some light is thrown on this phase of the subject by observations on plants low in nitrogen or nutrients. Suppose the plant has pro-

duced a large number of staminate buds. If a supply of nitrogen is suddenly available, the plant is forced to slough off these staminate buds and produce either pistillate flowers or tissue in the form of laterals, depending on the type of plant under observation.

Thus the data seem to point to a chromosome factor or factors for sex determination, even though environment, by changing the nutrient balance, can change the sex ratio within certain limits. These limits are either one or the other flower type or vegetative growth. It can not be said that environment causes a change from a staminate to a pistillate flower condition, but rather that it causes a change from one or the other types of flowers to leaf and stem tissue. Environment, therefore, does not determine the sex; it merely produces conditions which make possible the expression of potentialities in the plant.

#### SUMMARY

Environmental factors as they affect the production of pistillate and staminate flowers on cucumber plants were studied.

An abundance of light tends to increase the number of staminate flowers, within certain limits.

The reduction of light materially increases the number of pistillate flowers and decreases the number of staminate flowers.

The use of electric light at an intensity of 100 foot-candles has a marked effect in increasing the number of staminate flowers during days of minimum sunlight.

The presence of fruit exerts an inhibitory effect on the development of the plant as well as on the production of pistillate flowers.

Certain selections having a heavy production of pistillate flowers at the nodes of the main stem show an absence of staminate flowers. These selections under reduced light conditions produce false parthenocarpic fruits when pollination is not accomplished, and even when it is accomplished many of the fruits are not fertilized.

The heavy production of pistillate flowers on a pistillate type is associated with fasciation which causes the plants to become more or less dwarfed because of the clusters of pistillate flowers which take the place of the growing tips.

Preliminary data on the production and location of pistillate flowers on cucumber plants indicate that these characters are controlled by some chromosome mechanism.

#### LITERATURE CITED

- (1) ALLEN, C. E.  
1917. A CHROMOSOME DIFFERENCE CORRELATED WITH SEX DIFFERENCE IN SPHEROCARPOS. *Science* (n. s.) 46: 466-467.
- (2) ———  
1919. THE BASIS OF SEX INHERITANCE IN SPHEROCARPOS. *Amer. Phil. Soc. Proc.* 58: 289-316, illus.
- (3) ALSTERBERG, G., and HÅKANSSON, A.  
1926. ÜBER MANOILOFFS REAKTIONEN UND DIE MÖGLICHKEIT, MIT HILFE DIESER DAS GESCHLECHT ZU BESTIMMEN. *Biochem. Ztschr.* 176: [251]-265.
- (4) BLACKBURN, K. B.  
1923. SEX CHROMOSOMES IN PLANTS. *Nature* 112: 687-688, illus.
- (5) ———  
1924. THE CYTOLOGICAL ASPECTS OF THE DETERMINATION OF SEX IN THE DIOECIOUS FORMS OF LYCHNIS. *Brit. Jour. Expt. Biol.* 1: 413-430, illus.

- (6) BLARINGHEM, L.  
1922. ÉTUDES SUR LE POLYMORPHISME FLORAL. III. VARIATIONS DE SEXUALITÉ EN RAPPORT AVEC LA MULTIPLICATION DES CARPELLES CHEZ LE MERCURIALIS ANNUA L. Bul. Soc. Bot. France (ser. 4, t. 22) 69: 84-89, illus.
- (7) ———  
1923. ÉTUDES SUR LE POLYMORPHISME FLORAL. IV. SEXUALITÉ ET MÉTAMORPHOSE DES ÉPIS DE PLANTAGO LANCEOLATA L. Bul. Soc. Bot. France (ser. 4, t. 23) 70: 717-725, illus.
- (8) ———  
1924. SUR LE DIMORPHISME SEXUEL DES FLEURS ET LA VARIABILITÉ SPÉCIFIQUE. Bul. Soc. Bot. France (ser. 4, t. 24) 71: 265-273, illus.
- (9) BRINK, R. A.  
1924. THE PHYSIOLOGY OF POLLEN. I-IV. Amer. Jour. Bot. 11: 218-228, 283-294, 351-364, 417-436, illus.
- (10) BURBIDGE, F. W.  
1890. NUTRITION V. SEX. Gard. Chron. (3) 7: 25.
- (11) CAMUS, E. G.  
1904. NOUVELLE NOTE SUR LE SALIX HIPPOPHAEFOLIA THUILL. ET SUR LE S. UNULATA EHRRH. Bul. Soc. Bot. France (ser. 4, t. 4) 31: CXXXVII-CXLI. [Abstract in Gard. Chron. (3) 41: 57, 1907.]
- (12) CASTLE, R. L.  
1904. POMOLOGY AS A STUDY. Jour. Roy. Hort. Soc. 29: 146-160.
- (13) CLUTE, W. N.  
1925. CHANGING THE SEX IN PLANTS. Gard. Chron. Amer. 29: 18.
- (14) CORRENS, C.  
1908. WEITERE UNTERSUCHUNGEN UBER DIE GESCHLECHTSFORMEN POLYGAMER BLÜTENPFLANZEN UND IHRE BEEINFLUSSBARKEIT. Jahrb. Wiss. Bot. 45: [661]-700, illus.
- (15) CUMMINGS, M. B., and JENKINS, E. W.  
1923. STERILITY OF STRAWBERRIES; STRAWBERRY BREEDING. Vt. Agr. Expt. Sta. Bul. 232, 61 p., illus.
- (16) DAVEY, A. J., and GIBSON, C. M.  
1917. NOTE ON THE DISTRIBUTION OF SEXES IN MYRICA GALE. New Phytol. 16: 147-151, illus.
- (17) EMERSON, R. A.  
1924. A GENETIC VIEW OF SEX EXPRESSION IN THE FLOWERING PLANTS. Science (n. s.) 59: 176-182.
- (18) FUJII, K.  
1895. PHYSIOLOGICAL RESEARCHES ON THE SEXUALITY OF THE FLOWERS OF PINUS DENSIFLORA SIEBOLD ET ZUCCARINI. (Abstract) Gard. Chron. (3) 18: 428.
- (19) GARDNER, V. R.  
1923. STUDIES IN THE NUTRITION OF THE STRAWBERRY. Missouri Agr. Expt. Sta. Research Bul. 57, 31 p., illus.
- (20) GEORGESON, C. C.  
1910. HYBRID STRAWBERRIES. Alaska Agr. Expt. Stas. Ann. Rpt. 1909: 11-14, illus.
- (21) GUINIER, P.  
1921. VARIATIONS DE SEXUALITÉ, DIOÏCITÉ ET DIMORPHISME SEXUEL CHEZ LE PINUS MONTANA MILL. ET LE P. SYLVESTRIS L. (Abstract) Compt. Rend. Soc. Biol. [Paris] 84: 94-96.
- (22) HEIMLICH, L. F.  
1927. MICROSPOROGENESIS IN THE CUCUMBER. Natl. Acad. Sci. Proc. 13: 113-115.
- (23) IRMSCHER, E.  
1924. ÜBER EINE ABÄNDERUNG DES ZAHLENVERHÄLTNISSSES ZWISCHEN MÄNNLICHEN UND WEIBLICHEN BLÜTEN BEI DER MONOECISCHEN BEGONIA WALLICHIANA. Mitt. Inst. Allg. Bot. Hamburg 6: [149]-158, illus.
- (24) JANCHEN, E.  
1923. DAS VERHALTEN DER GESCHLECHTSFAKTOREN BEI DER EMBRYOSACK-BILDUNG DER BLÜTENPFLANZEN. Ztschr. Induktive Abstam. u. Vererbungslehre 31: [261]-267.

- (25) KOZHUKHOV, S. A.  
1925. KARYOTYPISCHE EIGENTÜMLICHKEITEN DER KULTIVierten CUCURBITACEAE. Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding) 14 (2): [89]-[96], illus.
- (26) KRAUS, E. J., and KRAYBILL, H. R.  
1918. VEGETATION AND REPRODUCTION WITH SPECIAL REFERENCE TO THE TOMATO. Oreg. Agr. Expt. Sta. Bul. 149, 90 p., illus.
- (27) MANOILOFF, E. O.  
1924. UEBER EINE CHEMISCHE REACTION BEI DER GESCHLECHTSBESTIMMUNG DER ZWEIFHÄUSIGEN PFLANZEN. Trudy Prikl. Bot. i Selekt. (Bul. Appl. Bot. and Plant Breeding) (1922/23) 13 (2): [503]-[505]. [In Russian. German résumé, p. [505].]
- (28) MEEHAN, T.  
1868. SEXUAL LAW IN ACER DASYCARPUM EHRB. Acad. Nat. Sci. Phila. Proc. 1868: 140-141.
- (29) ———  
1870. ON THE SEXES OF THE PLANTS [CONIFERS AND OTHER MONOEICIOUS TYPES]. Amer. Assoc. Adv. Sci. Proc. (1869) 18: 256-260.
- (30) MURNEEK, A. E.  
1926. EFFECTS OF CORRELATION BETWEEN VEGETATIVE AND REPRODUCTIVE FUNCTIONS IN THE TOMATO (LYCOPERSICON ESCULENTUM MILL.). Plant Physiol. 1: 3-56, illus.
- (31) NAGAI, I.  
1915. ON THE INFLUENCE OF NUTRITION UPON THE DEVELOPMENT OF SEXUAL ORGANS IN THE FERN PROTHALLIA. Jour. Col. Agr., Imp. Univ. Tokyo 6: [121]-164, illus.
- (32) RIEDE, W.  
1922. DIE ABHÄNGIGHEIT DES GESCHLECHTES VON DEN AUSSENBEDINGUNGEN. Flora (n. F. 15) 115: [259]-272.
- (33) ———  
1925. BEITRÄGE ZUM GESCHLECHTS- UND ANPASSUNGSPROBLEM. Flora (n. F. 18/19) 118/119: [421]-452.
- (34) SCHAFFNER, J. H.  
1922. THE SEXUAL NATURE OF VEGETATIVE OR DICHOTOMOUS TWINS OF ARISAEMA. Ohio Jour. Sci. 22: 149-154.
- (35) ———  
1922. CONTROL OF THE SEXUAL STATE IN ARISAEMA TRIPHYLLUM AND ARISAEMA DRACONTIUM [JACK IN THE PULPIT—DRAGON ROOT]. Amer. Jour. Bot. 9: 72-78.
- (36) ———  
1923. THE TIME OF SEX DETERMINATION IN PLANTS. Ohio Jour. Sci. 23: 225-240, illus.
- (37) ———  
1923. THE INFLUENCE OF RELATIVE LENGTH OF DAYLIGHT ON THE REVERSAL OF SEX IN HEMP. Ecology 4: 323-334, illus.
- (38) ———  
1925. SEX DETERMINATION AND SEX DIFFERENTIATION IN THE HIGHER PLANTS. Amer. Nat. 59: 115-127.
- (39) SHARP, L. W.  
1925. THE FACTORIAL INTERPRETATION OF SEX-DETERMINATION. Cellule 35: [193]-235, illus.
- (40) STOUT, A. B.  
1923. ALTERNATION OF SEXES AND INTERMITTENT PRODUCTION OF FRUIT IN THE SPIDER FLOWER (CLEOME SPINOSA). Amer. Jour. Bot. 10: 57-66, illus.
- (41) YAMPOLSKY, C.  
1919. INHERITANCE OF SEX IN MERCURIALIS ANNUA. Amer. Jour. Bot. 6: 410-442, illus.
- (42) ———  
1920. THE OCCURRENCE AND INHERITANCE OF SEX INTERGRADATION IN PLANTS. Amer. Jour. Bot. 7: 21-38.

# THE ANTIRACHITIC PROPERTIES OF COD-LIVER MEALS<sup>1</sup>

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## INTRODUCTION

In the production of cod-liver oil from the livers of the codfish (*Gadus callarias*) there remains a residue which in recent years has been dried and sold in the open market under the name "cod-liver meal." The nutritional value of this by-product of the cod-liver oil industry is creating a great deal of interest among poultrymen, poultry feed manufacturers, and animal feeders. It has been claimed that this liver residue, aside from the quality of its proteins, carries certain vitamin properties, principally those of an antirachitic nature.

It has been shown in the station laboratory, as well as by investigators<sup>2</sup> elsewhere, that cod-liver oils may vary greatly in their vitamin A and D content. This variation in vitamin potency is directly influenced by the amount of these factors present in the cod livers from which the oil is extracted. The method employed in the extraction of the oil may also exert a secondary effect on its vitamin content. Likewise, the residue remaining after the partial extraction of the fats would be expected to vary in vitamin properties, depending upon the original vitamin content of the livers, the amount of oil remaining in the residue, the method employed in expelling the oil, and the procedure used in drying the liver residue. Accordingly it appeared logical to inquire into the fat-soluble vitamin content of this product. The results obtained on the antirachitic properties of cod-liver meals form the basis for this report.

## EXPERIMENTS WITH CHICKS

The first experiments on the antirachitic properties of liver residues were conducted with chicks. For this purpose 156 day-old White Leghorn chicks hatched from eggs laid by hens of the same nutritional history were divided into 6 lots of 26 each. Each lot was confined in an indoor pen 3 by 6 feet. The only sunlight available was that which filtered through closed, muslin-covered windows.

Lot 1 received a ground mash of yellow corn 57 parts, wheat middlings 20, dried buttermilk 15, meat meal 5, calcium carbonate 2, and sodium chloride 1. Lots 2, 3, and 4 received a similar ration in which the 5 parts of meat meal were replaced by an equal quantity of a different cod-liver meal in each lot. The ration of lot 5 was the same as that of lot 1, with the addition of 2 parts cod-liver oil. Lot 6 received a ration in which liver meal No. 3 served as the main source of protein as well as the antirachitic factor. It had the following composition: Yellow corn, 65 parts; wheat middlings, 20;

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<sup>2</sup> HEUSER, G. F., and NORRIS, L. C. RICKETS IN CHICKS. I. VARIATIONS IN THE ANTIRACHITIC POTENCY OF DIFFERENT BRANDS OF COD-LIVER OIL. II. VARIATIONS IN THE ANTIRACHITIC POTENCY OF DIFFERENT GRADES OF COD-LIVER OIL. *Poultry Sci.* 6: 9-17, 94-98, illus. 1926-27.

cod-liver meal (No. 3), 12; calcium carbonate, 2; and sodium chloride, 1. All lots were cared for alike. The chicks were weighed individually every week. Pine shavings, changed weekly, were used as litter. Water was given as a drink.

The cod-liver meals used in the experiments were obtained from three different manufacturers and represented the dried liver residue remaining after the removal of the oil from fresh livers by the steam process. Meals 1 and 3 were of American manufacture; meal 2 was of Norwegian origin. Although no direct information as to the method and temperature employed in the manufacture of meals 1 and 2 was obtainable, it was apparent from the charred appearance of meal 2 that it had been subjected to a rather high temperature. Meal 1 in this respect presented a somewhat better appearance, but was darker in color than meal 3, which, according to the manufacturer, had been dried in a special vacuum drier at 70° F.

TABLE 1.—*The effect of feeding different cod-liver meals or cod-liver oil on the growth of chicks and the ash content of their tibiae*

Age of chicks (weeks)	Lot 1, fed basal ration (control)			Lot 2, fed ration containing 5 per cent cod-liver meal 1			Lot 3, fed ration containing 5 per cent cod-liver meal 2		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
	<i>Grams</i>			<i>Grams</i>			<i>Grams</i>		
1.....	55.9	26	0	52.7	26	0	52.2	26	0
2.....	75.2	26	0	69.6	26	0	68.1	26	0
3.....	104.4	26	17	108.3	26	2	100.9	26	10
4.....	132.5	26	26	146.6	26	7	131.1	26	18
5.....	160.5	25	25	201.1	26	16	158.4	26	26
6.....	189.8	25	25	238.4	26	18	175.6	26	26
Average per cent of ash in tibiae (fat free).....	32.23±.22			39.19±.69			32.32±.37		

Age of chicks (weeks)	Lot 4, fed ration containing 5 per cent cod-liver meal 3			Lot 5, fed ration containing 2 per cent cod-liver oil			Lot 6, fed ration containing 12 per cent cod-liver meal 3		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
	<i>Grams</i>			<i>Grams</i>			<i>Grams</i>		
1.....	49.6	26	0	50.3	26	0	53.6	26	0
2.....	67.7	26	0	74.8	26	0	69.8	26	0
3.....	109.0	25	0	113.1	26	0	100.3	26	0
4.....	163.0	25	0	160.9	26	0	132.3	26	0
5.....	221.8	25	0	218.7	26	0	155.9	26	0
6.....	268.1	25	1	279.6	26	0	202.4	26	0
Average per cent of ash in tibiae (fat free).....	44.14±.58			47.61±.35			47.58±.21		



The chicks in the first four lots were continued on the experiment for six weeks, when 12 representative birds from each of lots 1, 2, 3, and 4 were killed for bone analysis. At the same time six birds were taken from each of lots 5 and 6. The remaining 20 birds in each of the two last-mentioned lots were continued on the experiment to the eleventh week. For bone analysis the tibiae were removed, freed from adhering tissue, dried, crushed, then extracted with hot alcohol and ether, and subsequently ashed in an electric muffle furnace. The data are recorded in Table 1. The percentage of ash is expressed on the fat-free basis.

It is evident from the results secured (Table 1) that the three cod-liver meals varied markedly in their antirachitic properties. Meal 2, fed lot 3, exerted no antirachitic effect, as is attested by the number of cases of leg weakness and by the ash content of the tibiae. Meal 1 proved to be better from a calcification standpoint than meal 2—accounting for an approximate 7 per cent increase in ash content over the basal ration (lot 1) and liver meal 2 (lot 3). Although meal 3 proved the most efficient of the three liver residues—making for an increased ash content of approximately 12 per cent over the control group (lot 1) at a 5 per cent level of intake—it did not prove antirachitically equivalent to 2 per cent cod-liver oil (lot 5). Increasing the intake of meal 3 to 12 per cent (lot 6) resulted in an apparently normal ash content of the tibiae.

The growth of the chicks in all lots except 6 paralleled the vitamin-D intake. Meal 3 again proved superior to the other two meals, but not quite equivalent to 2 per cent cod-liver oil. The slower growth of lot 6 may in part be accounted for by the lowered protein intake. The 20 birds that remained in lots 5 and 6 at the sixth week and continued on the same rations weighed 718.7 and 408.5 grams, respectively, at the eleventh week. No cases of leg weakness were observed in either lot, and from all appearances the birds were all normal except for the retarded growth of lot 6. There were evidences of mild digestive disorders when the meal was fed at a 12 per cent level.

TABLE 2.—*Composition of the three cod-liver meals*

Sample	Moisture	Ash	Ether extract	Protein (N×6.25)
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Cod-liver meal 1.....	8.70	4.10	30.70	43.56
Cod-liver meal 2.....	5.85	3.25	23.21	54.18
Cod-liver meal 3.....	6.21	3.54	44.19	39.06

A chemical analysis of the three liver meals revealed a marked variation in their protein and residual fat content (Table 2). Meal 3 contained approximately twice as much ether-extractable material as meal 2, while meal 1 occupied an intermediate position. These facts suggested that the amount of protection which these meals were able to exert against leg weakness (experiment 1) was proportional to the residual fat content. To test out this hypothesis, 80-day-old White Leghorn chicks of the same nutritional history were divided into four lots of 20 each and fed a ration similar to that used in experiment 1, except that the different meals were so incorporated as to supply approximately 3 per cent of ether extract. To make

the rations fairly comparable as to total protein, the dried buttermilk was reduced accordingly. The rations had the following composition:

	Lot 1	Lot 2	Lot 3	Lot 4		Lot 1	Lot 2	Lot 3	Lot 4
Yellow corn.....	55	57	59	55	Cod-liver meal.....		10	13	7
Wheat middlings.....	20	20	20	20	Calcium carbonate.....	2	2	2	2
Dried buttermilk.....	15	10	5	15	Sodium chloride.....	1	1	1	1
Meat meal.....	5				Cod-liver oil.....	2			

Lot 2 received meal 1, lot 3, meal 2; and lot 4, meal 3.

All four lots were housed in separate 3 by 6 foot pens, located in the poultry building. Direct sunlight was not accessible. Planer shavings were used as litter. Fresh water was supplied daily.

TABLE 3.—*Effect on chicks produced by feeding cod-liver oil or by feeding different cod-liver meals on the same ether-extract basis*

Age of chicks (weeks)	Lot 1, fed ration containing 2 per cent cod-liver oil			Lot 2, fed ration containing 10 per cent cod-liver meal 1			Lot 3, fed ration containing 13 per cent cod-liver meal 2			Lot 4, fed ration containing 7 per cent cod-liver meal 3		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
1.....	<i>Grams</i> 51.9	20	0	<i>Grams</i> 50.9	20	0	<i>Grams</i> 50.0	20	0	<i>Grams</i> 53.3	20	0
2.....	70.9	20	0	76.9	20	0	63.5	20	0	79.2	20	0
3.....	119.2	20	0	110.7	20	0	87.8	20	0	114.2	20	0
4.....	169.5	20	0	153.2	20	0	107.1	19	6	152.3	20	0
5.....	222.5	20	0	191.7	20	0	121.9	18	12	194.5	19	0
6.....	292.3	19	0	256.3	19	4	147.3	16	16	256.0	19	0
Average per cent of ash in tibiae (fat free).....	48.97±32			41.47±64			37.07±27			47.08±42		

The chicks, as in the preceding test, were continued on the experiment for six weeks, when 10 representative birds from each lot were killed for bone analysis as before. The results are tabulated in Table 3. Meal 3, although fed at the lowest level, again proved the best antirachitically, followed in order by meals 1 and 2. The ash content of the tibiae from the groups fed cod-liver meal, lots 2, 3, and 4, was approximately from 2 to 5 per cent higher than that for the same lots in experiment 1. This somewhat better calcification may be explained on the basis of increased vitamin-D intake. The writers are inclined to believe, however, that the higher ash content, particularly in the case of lot 3, which received meal 2, was influenced by the increased phosphorus intake in establishing a more favorable calcium-phosphorus relationship for normal bone formation. Later work with rats substantiates the contention that the better calcification noted with meal 2 in experiment 2 was not due to increased vitamin-D intake. In general, the results of experiment 2 are in accord with those of the first experiment. They also show that the antirachitic properties of these meals bear no direct relation to their fat content.

## EXPERIMENTS WITH RATS

To obtain further knowledge of the antirachitic value of the different cod-liver meals, the writers used the rat as the experimental animal. The rat represents a rather rapidly growing animal, which can be kept under better nutritional control than the chick, and consequently affords better analytical results.

Bethke, Steenbock, and Nelson<sup>3</sup> pointed out some time ago that with the rat on low calcium rations the amount of cod-liver oil necessary to furnish the antirachitic factor in sufficient amounts varied inversely with the calcium content of the diet. The same conclusion was reached in the case of rats on relatively high phosphorus diets. Other unpublished work with this animal has convinced the writers that the ratio of calcium to phosphorus in the diet bears a direct relation to the antirachitic requirements of this species for normal calcification. Since cod-liver meal contains a relatively high percentage of phosphorus, it is obvious that the addition of this product to a rickets-producing ration would affect the calcium-phosphorus relation and make conditions more favorable for calcification. In order not to complicate the results by a change or adjustment in the calcium-phosphorus relation, the writers decided to use the extracts of these meals.

It was provisionally assumed that the major portion of the antirachitic properties of these liver meals was present in the residual fat, and therefore would be removed by a fat solvent like ether, as suggested by the work of Shipley, Kinney, and McCollum.<sup>4</sup> Although it is not safe to conclude from the work of these investigators that all of the antirachitic factor is extracted by ether, the vitamin-D content of the various extracts at least should be comparable if they are prepared under the same conditions.

The experiments with rats were carried out by feeding the cod-liver meal extracts at levels of 0.5 per cent in a rickets-producing ration. The ration employed was the one reported by Steenbock and his associates,<sup>5</sup> which consisted of yellow corn 76 parts, wheat gluten 20, calcium carbonate 3, and sodium chloride 1. The extracts were prepared by extracting the meals for 24 hours with ether in a large Soxhlet extractor. The excess ether was removed from the extract by distillation under reduced pressure. The resulting oily residue was then added directly to the ration.

The rats used were raised in the station laboratory under standardized conditions. They were taken at the age of 25 to 28 days, weighing from 50 to 64 grams. In all, 5 litters of 6 rats each were divided into 6 lots of 5 each. One lot was killed at the beginning of the experiment and the femurs removed for ash analysis. Another lot received the basal rachitic ration and served as a control. Three more lots were fed the rachitic basal mixture fortified with 0.5 per cent of the

<sup>3</sup> BETHKE, R. M., STEENBOCK, H., and NELSON, M. T. FAT-SOLUBLE VITAMINS. XV. CALCIUM AND PHOSPHORUS RELATIONS TO GROWTH AND COMPOSITION OF BLOOD AND BONE WITH VARYING VITAMIN INTAKE. *Jour. Biol. Chem.* 58: 71-103, illus. 1923.

<sup>4</sup> SHIPLEY, P. G., KINNEY, E. M., and MCCOLLUM, E. V. STUDIES ON EXPERIMENTAL RICKETS. XXIV. THE EFFECT OF CERTAIN EXTRACTS OF PLANT TISSUES ON FLORID RICKETS. *Jour. Biol. Chem.* 59: 165-175. 1924.

<sup>5</sup> STEENBOCK, H., HART, E. B., ELVEHJEM, C. A., and KLETZIEN, S. W. F. DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION. VI. THE ANTIRACHITIC PROPERTIES OF HAYS AS RELATED TO CLIMATIC CONDITIONS WITH SOME OBSERVATIONS ON THE EFFECT OF IRRADIATION WITH ULTRA-VIOLET LIGHT. *Jour. Biol. Chem.* 66: 425-440, illus. 1925.

ether extract of the different liver meals; the remaining lot received the basal mixture supplemented with 0.5 per cent crude medicinal cod-liver oil. The five groups of rats were fed for a period of four weeks, when they were chloroformed and the femurs removed for ash analysis. For this purpose the bones were freed from tissue, dried at 50° C. for 24 hours, crushed, then extracted with hot alcohol and ether for 36 hours, dried, and ashed in a muffle furnace. The results are recorded in Table 4.

TABLE 4.—*The calcifying properties of the ether extract of different cod-liver meals and of cod-liver oil fed to rats as an addition to a rickets-producing ration*

Lot No.	Addition to ration	Average initial weight	Average final weight	Average ash in femurs
		Grams	Grams	Per cent
1349	None (control lot, killed at beginning of experiment).....	57	89	39.32±.46
1354	None (rachitic control).....	58	89	27.03±.85
1350	0.5 per cent ether extract of cod-liver meal 1.....	58	89	29.50±.91
1351	0.5 per cent ether extract of cod-liver meal 2.....	56	90	26.78±.86
1352	0.5 per cent ether extract of cod-liver meal 3.....	58	87	38.19±.83
1353	0.5 per cent cod-liver oil.....	57	80	45.85±.39

\* Each lot contained 5 rats.

It is seen in Table 4 that over the four-week period the ash content of the femurs from rats in lot 1354, fed the unmodified rickets-producing ration, markedly decreased as compared to the average of 39.32 per cent for the control lot killed at the beginning of the experiment. The addition of 0.5 per cent of the ether extract of cod-liver meal 2 to the basal ration exerted no favorable effect on calcification. This observation substantiates the results obtained with chicks. The 0.5 per cent of the extract from meal 1 gave evidence of affording a slight antirachitic effect, but did not prove nearly as efficient as extract from meal 3. The latter, however, did not furnish sufficient vitamin D at a 0.5 per cent level to produce a normal ash content, as did 0.5 per cent cod-liver oil. The results secured with rats are in general agreement with those obtained with chicks.

To gain further knowledge of the calcifying properties of the extracts from cod-liver meals in comparison with the cod-liver oil previously used, the line-test method as described by McCollum and his coworkers<sup>6</sup> was employed. The rats used for these tests were animals of the writers' own breeding, started when from 25 to 28 days of age and weighing approximately 60 grams on the Steenbock rickets-producing ration previously described. After 24 days on this ration, when moderate rickets had developed in all rats, the diets were supplemented for 10 days with varying amounts of cod-liver oil and the oily extract of the meals thoroughly incorporated in the ration. At the end of this time the rats were killed with ether and the radii and ulnas removed and examined for calcium deposition by staining with silver nitrate.

<sup>6</sup> MCCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., and PARK, E. A. STUDIES ON EXPERIMENTAL RICKETS. XVI. A DELICATE BIOLOGICAL TEST FOR CALCIUM-DEPOSITING SUBSTANCES. Jour. Biol. Chem. 51: 41-49, illus. 1922.

TABLE 5.—*Calcium deposition in rachitic rats after the rickets-producing ration was supplemented by the ether-extract of different cod-liver meals or by cod-liver oil*

Supplement to rickets-producing ration	Rat No.	Weight	Average daily consumption	Calcium deposition <sup>a</sup>
		<i>Grams</i>	<i>Grams</i>	
Ether extract of cod-liver meal 1:				
0.40 gram.....	4434	73-87	6.5	—
	4439	80-80	6.5	—
0.60 gram.....	4435	75-89	7.0	—
	4441	71-79	7.0	—
1.00 gram.....	4437	81-92	7.0	—
	4443	80-88	7.5	+
Ether extract of cod-liver meal 2:				
0.60 gram.....	4438	93-100	8.0	—
	4446	83-86	7.0	—
1.00 gram.....	4442	69-75	7.5	—
	4447	74-77	5.5	—
2.00 grams.....	4444	79-90	8.0	—
	4449	82-83	6.5	—
ether extract of cod-liver meal 3:				
0.40 gram.....	4453	91-102	8.0	—
	4458	88-90	7.0	—
0.60 gram.....	4454	74-84	6.0	—
	4459	92-96	8.0	++
1.00 gram.....	4455	81-92	7.0	++
	4460	83-88	7.5	++
Cod-liver oil:				
0.10 gram.....	4448	80-81	7.0	++
	4456	85-99	8.5	+++
0.20 gram.....	4450	74-85	7.5	+++
	4452	74-80	6.0	+++

<sup>a</sup> — = No calcium deposition, + = evidence of calcium deposition; ++ = narrow line of calcium; +++ = wide line of calcium.

The results in part, as recorded in Table 5, show that the cod-liver oil used in all these experiments was at least 6 times as potent antirachitically as the extract from meal 3 and more than 10 times as active as the extract from meal 1. The oily extract of meal 2 apparently possessed very few if any antirachitic properties, which is in agreement with the results obtained in feeding the meal to growing chicks.

#### SUMMARY AND CONCLUSIONS

The results of experiments with chicks and rats show conclusively that the dried residues remaining after the extraction of oil from fresh cod livers vary markedly in their antirachitic properties. The antirachitic variation was not proportional to the residual fat content of the livers. Nor did the ether-extractable fraction prove nearly as potent as ordinary cod-liver oil. Accordingly, it would seem unwise to use the liver meal as an antirachitic substitute for a good grade of cod-liver oil in either poultry or livestock production.

Whether cod-liver meals may possess other merits aside from their questionable fat-soluble vitamin content remains to be determined.



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## A TREE CLASSIFICATION FOR THE SELECTION FORESTS OF THE SIERRA NEVADA<sup>1</sup>

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### INTRODUCTION

Individuality in man is accepted without question. In domestic animals, also, good and bad individuals are generally recognized. Even in some cultivated plants—orange trees and rubber trees—the poor producers are searched out and eliminated. Indeed, individual variability is a normal condition in all groups of organisms. Yet forest trees are rarely thought of in terms of the individual. Forest products are seldom of sufficient value to justify tending the individual tree. But there is no more reason why two western yellow pine trees should grow with equal rapidity or bear equal amounts of seed because they grow under identical conditions than that two men should attain equal strength or equal mentality because they receive the same food. When to inherent variability are added the effects of a wide range of interrelated environmental factors, the great differences in the behavior of individual trees can be readily appreciated. It is adjudged a common fault to lose sight of the forest through confusion of the trees. Much more frequently in forestry the mass effect is the more obvious, and there is failure to see in their proper relationships the elementary components of the forest—the individual trees.

### EXISTING TREE CLASSIFICATIONS

Thus far, within the species, foresters in the United States have necessarily been limited in the classification of trees to only slightly less generalized groups of individuals defined by differences in vigor or value. Dominant, codominant, intermediate, and suppressed, or some such classes based upon position in the crown canopy, are universally recognized. In European countries, where forestry has become most intensive, the necessity for distinguishing more clearly these differences in vigor and value has led several foresters to consider not only position in the crown canopy but crown development and stem form as well.

In Germany in 1884 G. Kraft formulated a tree classification based on crown development (6).<sup>2</sup> This was amplified in 1897 by C. R. Heck (4), who distinguished stem classes within each crown class. Marked differences in growth (5) and seed bearing (14) between these tree classes have been clearly determined from the study of sample plots over long periods.

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 770.

In 1902 German forest research institutions agreed upon a tree classification to be used in thinning research (9, p. 200). In Switzerland, France, Denmark, Finland, and Sweden the question of tree classes is also dealt with extensively in the literature of thinning. The late Gunnar Schotte in the publication of the Swedish State Forest Experiment Station in 1912 gave a review of tree classifications hitherto in use, and added one of his own based partly upon position in the crown canopy, partly upon crown development, and to a lesser degree upon stem form (12, 8).

#### THE NEED FOR A NEW CLASSIFICATION

All these classifications, so far as known, apply to even-aged stands, for the most part well stocked, and of comparatively simple composition. In the pine stands of California the situation is complicated by irregularity in age, understocking, and mixtures of several tolerant and intolerant species. In addition to position in the crown canopy, crown development, and stem form, the age of each tree must be considered in any grouping based on capacity for growth and seed production. The conventional crown classification applicable to even-aged stands, if recognized at all, is an unsatisfactory index of vigor under these conditions. In the western yellow pine stands of the Southwest some improvement has been effected by the recognition of two broad age classes—"blackjacks" and "yellow pines" (?). In California a rough segregation of age classes is now practiced in marking, but is recognized to be inadequate. Selection by diameter limits is a poor makeshift, to be used only when marking is not feasible.

The history of older Forest Service cuttings and examinations of marking on several of the most important recent sales indicate clearly that there is still lack of reasonable uniformity in the application of the same marking principles in similar stands. At the same time there is a lack of adaptability in applying the principles to varying conditions. Incorrect marking has frequently resulted in rates of growth much below the capacity of the site. Far too many unproductive trees are being retained.

The policy of the Forest Service in the California pine region is to reserve 20 to 30 per cent of the original stand in sound thrifty trees capable of good growth and likely to survive windfall, insect attacks, or fire, to make feasible a second cutting in comparatively inaccessible areas in reasonable time. A considerable portion of this reserve must be high-quality timber, and this necessarily means retaining rather large trees. As a source of seed in case the advance reproduction should be destroyed by fire, four or more seed trees per acre, also of rather large size, must be left. Far more skill is demanded in marking for a large reserve than for a heavy selection or seed-tree cutting. On many cuttings the provision for reservation of a certain percentage of the stand has been too strictly followed, with insufficient consideration for the condition of the stand or site variations. A recognized system of tree classification would no doubt result in uniformly better marking. As a basis for comparison of marking jobs in sales inspections such a system has obvious advantages.

Forest entomologists have demonstrated that the western pine beetle (*Dendroctonus brevicornis*) has a definite tendency to select



certain trees in endemic infestation.<sup>3</sup> A clear definition of the susceptible types of trees would permit their elimination by marking, and thus greatly reduce this important source of loss.

In studies of sample plots in selection stands where records are made of individual trees, a uniform system of tree classification is needed to simplify recording and permit accurate comparison of one area with another. E. J. Hanzlik has suggested using the Swedish system developed by Schotte for such work (3). For sample plots in even-aged stands this system doubtless works well, but omission of the age factor makes it unsuitable for application in selection stands or in cut-over areas.

A workable tree classification also offers interesting possibilities in appraisals, in marking to maintain certain standards of growth, in predicting future yields, in studies of susceptibility to fire damage, and in many other ways in which simple crown classes are now used in even-aged forests.

Whatever system of tree grouping is used in marking, it is not to be expected that there will be perfect agreement between different men. Border-line trees will be encountered in this work, just as they are in applying the accepted classifications for even-aged stands. Adherence, however, to a definite system of appraising each tree, based on easily discernible characteristics, will prevent obvious mistakes in marking, and so will raise the average rate of growth in cut-over stands, decrease losses, and improve the quality of seed trees. Agreement on a well-defined terminology is essential to mutual understanding.

#### BASIS FOR CLASSIFICATION

The conclusions presented here are the results of 15 years' observation of more than 20,000 numbered trees in 25 permanent sample plots covering about 300 acres, established on typical sale areas in the Sierra. Detailed crown and bole descriptions permit segregation of the trees into the classes to be described below. On this basis comparisons of growth and seed bearing have been made from measurements taken at five-year intervals. For the sake of brevity, the results for only one species, western yellow pine (*Pinus ponderosa*), are given. This species occurs on all the plots in numbers sufficient to give a reasonably good basis of data. It is widely known, and is more easily grouped into the proposed classes than any of the other species.

The proposed classification represents an effort to segregate into groups the trees with certain combinations of characteristics that are known from previous studies (1, 2) to have similar influences on growth or seed bearing. It is obviously impossible to consider each of the interrelated variable elements singly. The significance of any one factor can not be isolated. Practice demands that the number of classes be small and that the factors on which they are based be readily distinguishable. In actual field tests of this system no serious difficulties have been encountered by the men, even though they had little previous knowledge of the grouping.

The major factors considered in the make-up of these classes are:

1. Four general age groups—young (less than 50 years), thrifty mature (50 to 150 years), mature (150 to 300 years), and overmature (over 300 years).

<sup>3</sup> Carefully controlled experiments have recently been conducted by H. L. Person at North Fork, Calif., on behalf of the Bureau of Entomology. A report of these studies is in preparation for publication in the Journal of Forestry.

2. Degree of dominance within these age groups, expressed in terms of the conventional crown classes—isolated, dominant, codominant, intermediate, and suppressed.

3. Crown development.

4. A supplementary estimate of thrift designated in three degrees of vigor—good, moderate, and poor.

The estimate of vigor is based on apparent age, degree of dominance, crown development, and, in addition, the density and color of the foliage, the form of the top (whether pointed, round, or flat), the size attained in relation to age, the color, thickness, and texture of the bark, and freedom from disease.

In marking, only sound, well-formed trees need be considered in such a classification. It is deemed undesirable to introduce complications in the form of subclasses for the multitude of defects which may possibly occur. The question of merchantability assumes priority and should be considered separately on the basis of already well-established criteria. Trees that are malformed, injured, or diseased should be removed from the stand wherever possible, and it is unnecessary to go further in segregating them by thrift classes.

In working up this material an effort was made to determine to what extent mechanical injuries and defects, such as fire scars and logging scars on the stem, fire or logging damage to the crown, broken or dead tops, etc., affect growth. The difficulty of isolating the effects of a given class of defects is apparent. On cut-over areas the number of defective trees has naturally been reduced, so that after division into comparable groups there is insufficient material to be of much significance. Damage to the crown that materially reduces the leaf area is usually reflected in a reduction in the rate of growth. In the present data no consistent relation is discernible between mechanical injuries to the stem and deficient vigor. Such injuries, when of sufficient extent to affect growth materially, should influence marking through predisposition to bring about losses and because of their effect on merchantability, rather than through their effect on growth. For purposes of study it is a simple matter to supplement the classification for sound trees with a description of defects that will permit elimination of uncertain influences from the data.

#### DESCRIPTION OF CLASSES

Seven tree classes are proposed, as follows (see fig. 1).

Class 1: *Age class*, young or thrifty mature; *position*, isolated or dominant (rarely codominant); *crown length*, 65 per cent or more of the total height; *crown width*, average or wider; *form of top*, pointed; *vigor*, good.

Trees of this class are rarely over 30 inches in diameter even on good sites. The bark is dark brown and roughly fissured into ridges or small plates. The foliage is rich green in color and dense, owing to retention of the needles of three to five seasons or more, except at the base of the crown. The needles are often long and coarse, especially near the top. Terminal buds are large. The top is pointed, owing to the rapid elongation of the terminal. Thrifty open-grown young trees belonging to this class are, however, sometimes round topped because of excessive lateral growth of branches near the top. On the other hand, slow-growing trees sometimes have pointed tops, due to weak development of laterals. The annual whorls of branches

and internodes are still distinct, except in the lower crown. Branches are horizontal or upward curving, except at the base of the crown where suppression is taking place. Numerous stubs of dead branches are likely to be present below the crown.

Class 2: *Age class*, young or thrifty mature; *position*, usually codominant (rarely isolated or dominant); *crown length*, less than 65 per cent of the total height; *crown width*, average or narrower; *form of top*, pointed; *vigor*, good or moderate.

Such trees are usually less than 24 inches in diameter. They are commonly the inside codominant trees of groups. The crowns are smaller and less dense than in trees of the first type. Otherwise they are similar to those of class 1.

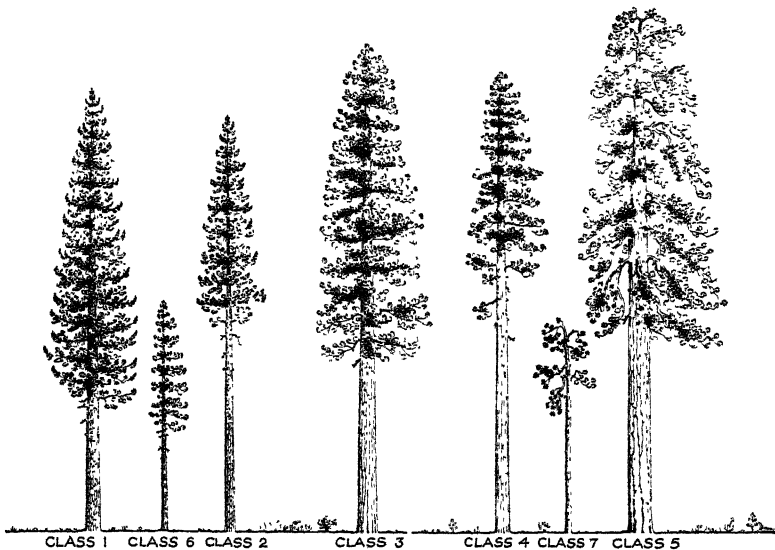


FIG. 1.—Classes 1, 2, and 6 represent young or thrifty mature trees; classes 3 and 4 mature trees; classes 5 and 7, mature or overmature trees. Form of top, crown width and length, and position in the crown cover are other determining characteristics

Class 3: *Age class*, mature; *position*, isolated or dominant (rarely codominant); *crown length*, 65 per cent or more of total height; *crown width*, average or wider; *form of top*, round; *vigor*, moderate.

These trees are ordinarily between 18 and 40 inches in diameter, depending on site quality. The bark is light brown or yellow, with moderately large smooth plates. The foliage is less dense than in class 1 trees. The top is round, because of slow height growth. The nodes are indistinct, because of incomplete whorls of branches. The branches are nearly all horizontal or drooping.

Class 4: *Age class*, mature; *position*, usually codominant (rarely isolated or dominant); *crown length*, less than 65 per cent of the total height; *crown width*, average or narrower; *form of top*, round; *vigor*, moderate or poor.

These are commonly the inside or codominant trees of this age class. Except for their small poorly developed crowns and smaller size, they are similar to class 3 trees.

Class 5: *Age class*, overmature; *position*, isolated or dominant (rarely codominant); *crown* of any size; *form of top*, flat; *vigor*, poor.

These are usually the largest trees in the stand. The bark is light yellow in color, the plates often very wide, long, and smooth, especially near the base. The bark may be thin, having weathered more rapidly than it has grown. The foliage is usually rather pale green and very thin. The needles are fairly short, appearing as tufts on the ends of the twigs. The needles of two or three seasons only may be retained, even near the top. The top is flat, the terminal rarely discernible. There is no appreciable elongation of the main axis. Scarcely any nodes are distinguishable. Nearly all the branches are drooping, gnarled, and crooked.

Class 6: *Age class*, young or thrifty mature; *position*, intermediate or suppressed; *crown* of any size, usually small; *form of top*, round or pointed; *vigor*, moderate or poor.

These are understory trees, rarely over 12 or 14 inches in diameter. The bark is dark and rough. The top is round or pointed, showing that some height growth is taking place. Whorls of branches are evident, though the internodes are short.

Class 7: *Age class*, mature or overmature; *position*, intermediate or suppressed; *crown* of any size, usually small; *form of top*, flat; *vigor*, poor.

These understory trees are rarely over 18 inches in diameter. The bark is light in color, thin, and smooth. The top is flat, the terminal rarely distinguishable. The foliage is excessively thin. The few branches present are gnarled and drooping.

The similarities and differences between these groups are perhaps more evident in the abbreviated comparison given in Table 1.

#### RELATIVE IMPORTANCE OF CLASSES IN THE STAND

The relative importance of the tree classes in the original and remaining stand and the proportion of the cut supplied by each are shown in Table 2, which summarizes the data from a typical stand in the Stanislaus National Forest cut over in 1923. Marking was carefully done by a marking board to conform as nearly as possible to existing cutting policy.

TABLE 1.—Summary of tree classification by system proposed <sup>a</sup>

	Age class	Crown class (position)	Crown length	Crown width	Form of top	Vigor
Class 1.....	Y-TM	X-D (C)	<i>Per cent</i> 65+	M-W	△	V
Class 2.....	Y-TM	C (X-D)	65-	M-N	△	V-M
Class 3.....	M	X-D (C)	65+	M-W	△	M
Class 4.....	M	C (X-D)	65-	M-N	△	M-P
Class 5.....	OM	X-D (C)	All.	All.	△	P
Class 6.....	Y-TM	I-S	All.	All.	△ (Λ)	M-P
Class 7.....	M-OM	I-S	All.	All.	△ (Λ)	P

<sup>a</sup> Significance of symbols is as follows:

Age class: Y-TM=young or thrifty mature; M=mature; OM=overmature.

Crown class: X=isolated; D=dominant; C=codominant; I=intermediate; S=suppressed.

Crown width: M=average; W=wider than average; N=narrower than average; All=any size.

Vigor: V=good; M=moderate; P=poor.

TABLE 2.—Volume and number of trees per acre and percentage of total volume in each tree class in original stand, in portion marked for cutting, and in reserved portion <sup>a</sup>

## VOLUME PER ACRE

Tree class	Original stand		Marked for cutting		Reserved		Percent- age of each class marked
	Board feet	Per cent	Board feet	Per cent	Board feet	Per cent	Per cent
Class 1.....	12,772	16.6	4,373	6.9	8,399	62.8	34.2
Class 2.....	2,259	2.9	353	.6	1,906	14.2	15.6
Class 3.....	21,297	27.8	18,407	29.1	2,890	21.6	86.4
Class 4.....	1,708	2.2	1,708	2.7	-----	-----	100.0
Class 5.....	37,875	49.4	37,875	59.7	-----	-----	100.0
Class 6.....	211	.3	51	.1	160	1.2	24.2
Class 7.....	595	.8	575	.9	20	.2	96.6
Total.....	76,717	100.0	63,342	100.0	13,375	100.0	82.6

NUMBER OF TREES PER ACRE <sup>b</sup>

Tree class	Original stand		Marked for cutting		Reserved		Percent- age of each class marked
	Number	Per cent	Number	Per cent	Number	Per cent	Per cent
Class 1.....	34.2	34.4	3.4	15.9	30.8	39.5	9.9
Class 2.....	7.6	7.7	.3	1.4	7.3	9.4	3.9
Class 3.....	7.3	7.3	6.4	29.9	.9	1.2	87.7
Class 4.....	.5	.5	.5	2.3	-----	-----	100.0
Class 5.....	8.2	8.3	8.2	38.4	-----	-----	100.0
Class 6.....	37.4	37.6	.2	.9	37.2	47.6	.5
Class 7.....	4.2	4.2	2.4	11.2	1.8	2.3	57.1
Total.....	99.4	100.0	21.4	100.0	78.0	100.0	21.5

<sup>a</sup> Board-foot figures give stands per acre for trees 12 inches diameter breast high and over, Scribner Decimal "C" rule. Stanislaus plot 5, cut in 1923.

<sup>b</sup> Figures show number of trees per acre 4 inches diameter breast high and over.

Classes 1, 3, and 5, the large-crowned dominant trees in the three general age groups, here form 93.8 per cent of the original board-foot volume for trees 12 inches in diameter and larger. Classes 3 and 5, mature and overmature dominants, provided 88.9 per cent of the cut. These tree classes contain the highest grade material (11), and probably represent more than 95 per cent of the present value of the entire stand. The class 1 trees, immature dominants, which formed 16.6 per cent of the original stand, supplied but 6.9 per cent of the volume cut. Such trees were marked only when it was necessary to thin groups of trees, to facilitate logging, or when they had been badly injured by removal of other trees. Classes 1 and 2 make up 77.1 per cent of the 13,375 board feet per acre reserved, representing very little present value, since they contain comparatively little high-grade lumber. Class 3 trees represent 21.6 per cent of the reserve volume, providing a few larger seed trees and some high-grade material for a second operation.

The small-crowned dominant and codominant trees of classes 2 and 4 and the intermediate and suppressed trees of classes 6 and 7 never form more than a small part of the merchantable volume. In number of trees, however, they are often relatively important. They are of primary interest because of their influence on future yields. The numerical importance of the tree classes in the above stand is

also shown in Table 2, with the inclusion of unmerchantable trees between 4 and 12 inches. In this stand, where there was an average of 99.4 trees per acre, most of the trees were in classes 1 and 6—34.2 per cent and 37.4 per cent, respectively. Classes 2, 3, and 5 were nearly equally represented by 7 to 8 per cent of the total. There were relatively few trees in classes 4 and 7.

### COMPARISONS OF GROWTH

For the sake of simplicity all the following growth comparisons are in terms of basal area, expressed as average annual rates per cent for the 15-year period. In terms of volume growth the differences shown would be accentuated, since the mature and overmature trees make practically no height growth. The size grouping is based on the diameters at the beginning of the period.

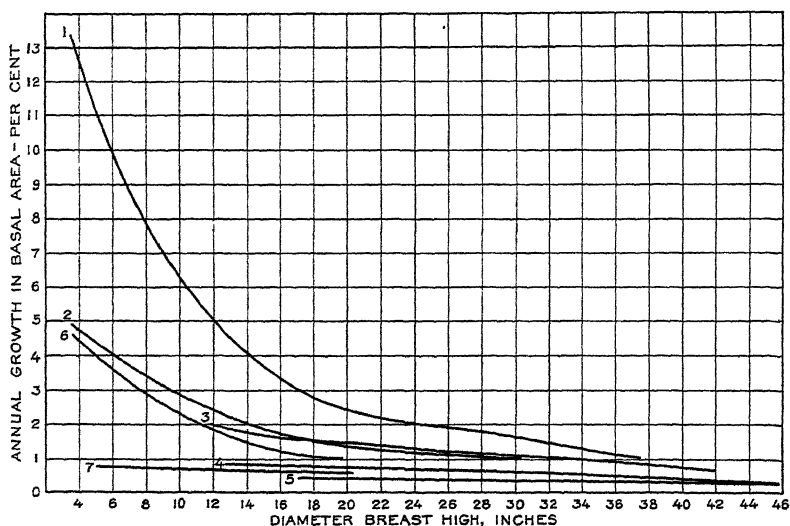


FIG. 2.—Annual basal area growth per cent, by tree classes, western yellow pine, site I (1,183 trees, 1910-1925)

Figure 2 permits comparison of the relative rates of growth of the seven tree types by diameter classes. The graph is based on 1,183 trees remaining on a first-quality site that was cut over in 1910, in the Stanislaus National Forest.

Class 1 trees are superior to the others for all diameters, but especially so in the smaller sizes. Class 2 trees have grown considerably more slowly than those of the first group, but in the smaller size classes are considerably above the trees in the remaining groups. Above 18 inches class 2 and 3 trees have grown at about the same rate, the larger crown area of the class 3 trees offsetting their greater age. The rate for class 3 shows little variation with size.

The trees of classes 4, 5, and 7 show still less variation with diameter, growing at a hopelessly slow rate for all sizes. The younger trees of class 6 give some promise, and the fact that few of them can be cut under present economic conditions is not a serious matter.

It is evident that a good rate of growth can not be expected from mature and overmature trees of classes 3, 4, 5, and 7, regardless of diameter, even on the best sites. Above 28 or 30 inches, differences

in rate of growth between the seven groups become unimportant. For these larger trees the risk of loss and seed-bearing ability should govern the choice in marking.

It is apparent from Figure 2 that age is the most important factor to be considered. Crown development and crown class differences are far more important in the young and submature than in the mature and overmature age classes.

A summary of the growth produced by this same stand is given in Table 3. Class 1 trees, which represented but 29.4 per cent of the residual stand, produced 57.7 per cent of the total growth and maintained the highest annual rate, 3.05 per cent. Class 2 trees grew only half as rapidly. Class 3 trees have not justified their retention from the standpoint of growth, and yet the annual rate of nearly 1 per cent is fairly good, and increase in value justifies reserving a considerable proportion of such trees. Little can be said in defense of the reservation of nearly one-fourth of the stand in classes 4 and 5.

TABLE 3.—Percentage of total basal area in 1910 and total growth 1910–1925 represented by each tree class, with annual growth rate (volume) maintained by each class over the 15-year period <sup>a</sup>

Tree class	Total basal area, 1910	Growth produced, 1910–1925	Annual growth rate
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Class 1.....	29.4	57.7	3.05
Class 2.....	11.4	11.2	1.53
Class 3.....	27.9	17.8	.98
Class 4.....	5.1	1.9	.59
Class 5.....	18.8	4.3	.35
Class 6.....	3.7	5.6	2.34
Class 7.....	3.7	1.5	.65
Total.....	100.0	100.0	1.56

<sup>a</sup> Western yellow pine, Stanislaus plots 2, 3, and 4. Site I. Cut over in 1910.

A similar relationship is shown graphically for a much larger area in Figure 3.

It should be remembered that the foregoing growth comparisons include the acceleration due to release by cutting, which should have culminated within 15 years after the thinning. The trees which were subordinate in the original stand have not generally improved sufficiently to equal the rates maintained by former dominants. It is usual for higher classes to decline to subordinate classes, but extremely exceptional for the reverse process to occur. Acceleration of growth is a minor consideration in the prevailing type of cutting with its tendency toward grouping of reserves. It is better to reserve trees already dominant than to rely upon the enhancement of increment in understory trees. This is particularly true in stands where the merchantable trees are all comparatively old. This point should be carefully considered in thinning groups.

#### COMPARISONS OF LOSSES

The ultimate success of marking is dependent upon both potential growth rates, as discussed above, and survival of the growing stock.

On the plots from which the data in Table 4 were taken there were 4,669 living trees, 4 inches in diameter and over, after cutting in 1910.

Between 1910 and 1925, 172 trees died. The distribution by classes of the total trees in 1910 and of the trees which died is shown in number of trees and basal area in Table 4. The fourth and last columns of figures show the relative liability of the different classes to loss, or the ratio of occurrence in the losses to occurrence in the stand.

On a numerical basis it is evident that the class 1 trees are the lowest risk. They are represented in the losses only about one-seventh as frequently as in the total stand. The liability to loss is from five to fifteen times greater for the other classes. Class 7 has the highest risk factor, followed by classes 4, 2, 6, 5, and 3. It is fortunate for the reserve policy that the class 3 trees have the lowest risk factor of any class except the first.

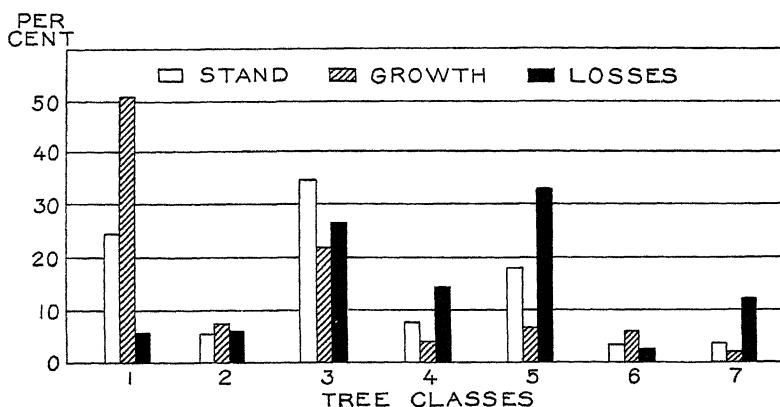


FIG. 3.—Apportionment between the seven tree classes of the total basal area of the stand, the total growth produced between 1910 and 1925, and the total loss in the same period. The data are taken from 4,669 trees totaling 7,855.64 square feet in basal area.

TABLE 4.—Apportionment of stand and mortality in each tree class, 1910–1925, in percentage of number of trees and basal area, with relative liability to loss of the trees in each class<sup>a</sup>

Tree class	Apportionment in trees			Apportionment in basal area		
	Total stand	Mortality	Ratio mortality to stand <sup>b</sup>	Total stand	Mortality	Ratio mortality to stand <sup>b</sup>
	Per cent	Per cent		Per cent	Per cent	
Class 1.....	34.0	4.7	0.14	24.9	5.2	0.21
Class 2.....	8.4	13.4	1.60	5.7	5.6	.98
Class 3.....	15.4	11.6	.75	35.0	26.7	.76
Class 4.....	6.6	11.6	1.76	7.8	14.5	1.86
Class 5.....	5.5	6.4	1.16	19.5	33.6	1.72
Class 6.....	16.5	23.2	1.41	3.2	2.3	.72
Class 7.....	13.6	29.1	2.14	3.9	12.1	3.10
Total.....	100.0	100.0	-----	100.0	100.0	-----

<sup>a</sup> Based on 4,669 trees having a total basal area in 1910 of 7,855.64 square feet. Mortality was 172 trees, totaling 334.99 square feet in 1910.

<sup>b</sup> Loss ratios, or risk factors, were obtained by dividing the figures in column 3 by those in column 2 and those in column 6 by those in column 5, class by class.

The significance of the loss data is more clearly brought out through basal area comparisons. On this basis the class 1 trees still represent much the lowest risk, followed by classes 6, 3, 2, 5, 4, and 7. The



greatest actual losses occurred in classes 3, 4, and 5, partly because the trees in these groups were large and formed a considerable portion of the reserve and partly because of a higher loss rate, especially in classes 4 and 5, as indicated by the high ratios in the last column of Table 4. (See also fig. 3.) If the relative risks were expressed in terms of value exposed to loss rather than basal area, the disparity would be much greater, since classes 3, 4, and 5 produce the highest percentage of upper-grade lumber.

The greatest single cause of mortality was bark beetles (*Dendroctonus*), which killed 61, or 35 per cent, of the 172 trees and accounted for 50 per cent of the basal-area loss. The distribution of insect losses by tree classes as compared with losses from other causes is given in Table 5.

Only a small part of the loss from insects occurred in the younger tree classes 1, 2, and 6, the greatest portion, nearly half the total, being in class 5. The relative risk of loss from insects in the various classes may be expressed by the ratios in Table 5, derived by dividing the percentage of the total basal-area loss occurring in each class by the percentage of the total basal area of the stand in each class. For comparison, similar ratios for other causes of loss are also shown.

TABLE 5.—*Distribution of losses in basal area from insects and other causes by tree classes, with relative liability to loss of the trees in each class*

Tree class	Insect damage		Other loss	
	Distribution of loss	Ratio mortality to stand	Distribution of loss	Ratio mortality to stand
	<i>Per cent</i>		<i>Per cent</i>	
Class 1.....	3.4	0.14	7.0	0.28
Class 2.....	5.4	.95	5.8	1.02
Class 3.....	14.6	.42	38.6	1.10
Class 4.....	16.2	2.08	12.8	1.64
Class 5.....	48.7	2.50	18.6	.95
Class 6.....	1.1	.34	3.5	1.09
Class 7.....	10.6	2.72	13.7	3.51
Total.....	100.0		100.0	

The probability of insect loss is greatest in class 7. Such trees appear 2.72 times as frequently in the losses as they do in the stand as a whole. The high factors for classes 4 and 5 are particularly significant because of the high-grade material contained in trees of these types. The above comparisons indicate that only trees of classes 4 and 5 are more subject to losses from beetles than from other causes. For class 5 trees the risk from beetles is more than two and one-half times as great as from other causes. It is worth noting that class 3 trees, which should properly make up the bulk of the better quality of timber reserved, are apparently less liable to damage from insects than from other causes. These relationships are shown graphically in Figure 4.

The above comparisons in basal area show the combined results of relative susceptibility and amount of timber exposed to loss. The selective tendency alone is more strikingly indicated by numerical comparisons, as shown graphically in Figure 4. The small-crowned mature codominants of class 4 are indicated to be the most liable to insect damage.

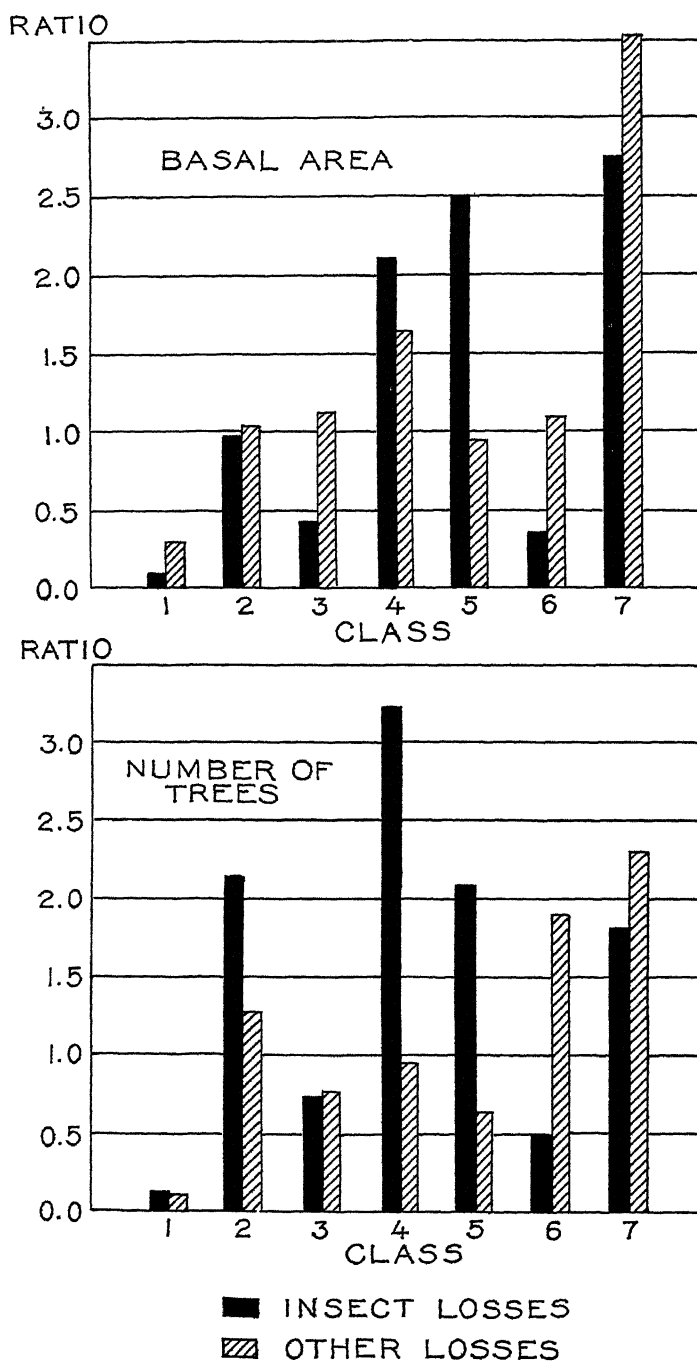


FIG. 4.—Ratio of apportionment of insect and other loss between tree classes to apportionment of stand between classes; in other words, the relative liability to loss of the different classes

The relative susceptibility of various tree types to killing by the western pine beetle, as indicated above, is in close agreement with the carefully controlled experiments by H. L. Person, of the Bureau of Entomology, already referred to. The elimination of susceptible trees in cutting would doubtless lessen endemic insect damage, the most important cause of loss on cut-over areas.

### COMPARISONS OF SEED BEARING

The seed-bearing capacity of forest trees has been shown by many investigators to be affected by a large number of environmental phenomena and inherent qualities. In the present study the aim has been to determine only how well the proposed tree classes integrate seed-bearing capacity, and no attempt has been made to record the more fundamental biological influences involved. Even so, the present results are but tentative.

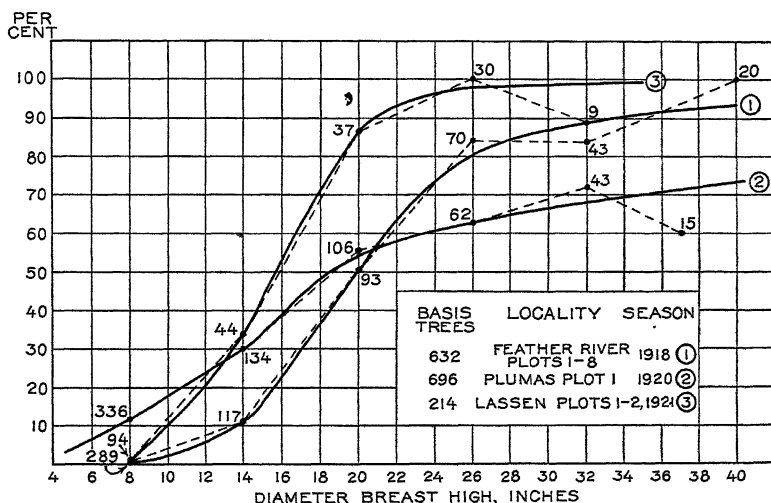


FIG. 5.—Percentage of trees of various diameters bearing cones, taken in three different localities in good average years. Data are for western yellow pine only

The physical difficulties encountered in a quantitative determination of the amount of seed borne are obvious. Unfortunately, too, there have been no really heavy general seed crops since the trees have been under observation, such as would permit comparison of seed bearing by similar tree types under different site conditions, or the consistency of bearing by the same trees from year to year. The data available necessarily limit comparisons to single localities and certain years. There are, however, certain outstanding differences which justify consideration. At present it is impossible to go further than to indicate what types of trees appear to be the best seed bearers. Determination of the exact quantity and quality of seed that is produced and remains undestroyed by insects, rodents, etc., must be left for further study.

Observation indicates that occasionally a potentially good seed bearer sets a heavy crop of fertile cones all of which are destroyed before maturity by cone beetles or rodents. For all species except

sugar pine (*Pinus lambertiana*) it has been found impossible to count the immature cones early enough to avoid these losses. In the absence of exact knowledge it is assumed for the present that such damage is not restricted to any particular tree class and that the figures presented are therefore comparable.

Under similar conditions of site, the major factors influencing seed bearing appear to be age, position in the crown canopy, and crown development. Since the size attained is closely correlated with age, crown class, and crown development, it is to be expected that there exists a close relationship between diameter and seed-bearing capacity. This relationship is shown in Figure 5 for three localities in different years.

Below 8 inches a negligible percentage of the trees bore cones. Between 8 and 26 inches the proportion of trees bearing cones increased rapidly. Above 26 inches practically all the trees bore some cones. At 20 inches, the size ordinarily regarded as representing satisfactory seed trees (13), from 50 to 90 per cent of the trees bore seed in these years.

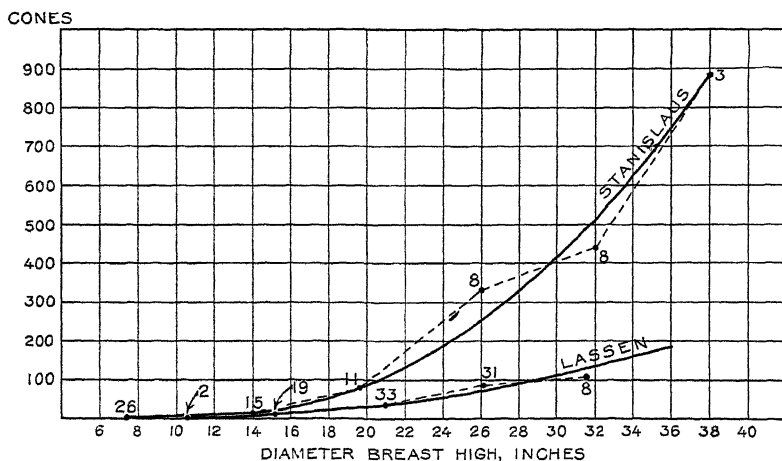


FIG. 6.—Number of cones per tree at various diameters. Count taken on western yellow pine on Lassen plots 1 and 2, and Stanislaus plot 5, 1925

It is well known that for many species there is a fairly definite alternation of seed years and barren years. Thus in the sample plots on the Lassen National Forest, only 62.5 per cent of the trees bore seed in both the seasons of 1921 and 1926. Furthermore, in the seasons of record the seed crops were never exceptional. Were records for a number of years available, covering one or more years of heavy crops, they would doubtless show much higher percentages of trees capable of bearing seed than the chart indicates.

The number of cones borne also varies consistently with diameter, which serves as an indirect integration of age and other factors. The number of cones per tree for two areas is shown in Figure 6 for the year 1926. The difference between the two curves illustrates the great local variation in the seed crop of the same season, the Lassen area being situated 160 miles north of the Stanislaus. These curves indicate that, in years of ordinary seed crops at least, rather large trees are necessary for the production of considerable quantities

of seed. Although 50 per cent or more of the 20-inch trees may be capable of bearing seed, the number of cones borne by them is relatively small.

The foregoing discussion indicates that the best seed trees will be found in the tree classes represented by the larger sizes. The summary in Table 6 of typical data from the Feather River group of plots makes this clearer.

The indicated differences are doubtless a result of the combined influences of age, crown development, and position or size. Comparison for a given size class is impossible because, in the nature of things, there is no one size class in which all the tree classes are well represented. It is apparent, however, that seed-bearing trees will most frequently be found among the principal trees of classes 1, 3, 4, and 5, and that classes 2, 6, and 7 provide an insignificant number of seed bearers. This relationship has been well established by a considerable number of investigations elsewhere (14, 15).

TABLE 6.—Percentage of trees in each class bearing cones and average diameter breast high of each class; eight Feather River plots, 1918

Tree class	Trees recorded	Trees bearing cones	Average class d b. h.
	Number	Per cent	Inches
Class 1.....	214	22.9	15.0
Class 2.....	84	14.3	13.5
Class 3.....	154	55.8	25.7
Class 4.....	52	40.4	20.4
Class 5.....	4	100.0	38.8
Class 6.....	97	1.0	7.3
Class 7.....	27	11.1	11.4
Total.....	632	27.9	-----

As to quantities of seed per tree, the scanty data from present observations and the more ample results of other investigators (10) leave little doubt that trees of the types found in classes 2, 6, and 7 bear but few cones. The largest numbers of cones per tree thus far counted were for trees of class 3.

#### SUMMARY

A tree or thrift-class grouping applicable to selection stands is needed which, unlike the conventional crown-class and other tree classifications for even-aged stands, will be suitable for all-aged mixed forests. On the basis of observations of permanent sample plots over a period of 15 years a tree grouping is proposed comprising seven classes defined by combinations of easily observed factors influencing vigor. The major factors considered are age, degree of dominance, and crown development. Confirmatory indications of relative vigor considered are form of top, color and density of foliage, character of bark, size, etc. Trees should be judged undesirable on the basis of unmerchantability and liability to loss rather than rate of growth.

Marked differences in rates of growth and susceptibility to loss demonstrate that the grouping proposed is a reliable integration of vigor.

Class 1 trees grow at the best rate and have the lowest loss liability factor. They are the least susceptible to insect attacks. They are good seed bearers when of sufficient size. The present value of timber from such trees is comparatively low. They should practically always be retained when sound.

Class 2 trees make fair growth, but are rather liable to loss and are poor seed bearers. They should be marked in preference to the larger class 1 trees when there are sufficient other thrifty trees to make up the reserve, or in thinning groups.

Class 3 trees grow rather slowly, but can be expected to increase in value without great risk of loss. They are good seed bearers. Such trees are desirable for retention as seed trees, or to constitute a moderate reserve of high-quality material for a second cutting in a reasonably short time. The usual tendency is to retain too many trees of this type. However, where sufficient class 1 trees are lacking, where good seed trees are needed, or where a second cutting must be provided for, there should be no hesitancy in leaving class 3 trees. Care should be taken not to include in this class overmature, slow-growing trees. Size varies greatly with site, but only exceptionally will trees of this type be found over 30 inches in diameter.

Classes 4 and 5 trees produce practically no growth even on the best sites. Their liability to loss is high. Their retention involves a large and insecure investment in high-quality timber. They should always be cut, unless there are no other available seed trees.

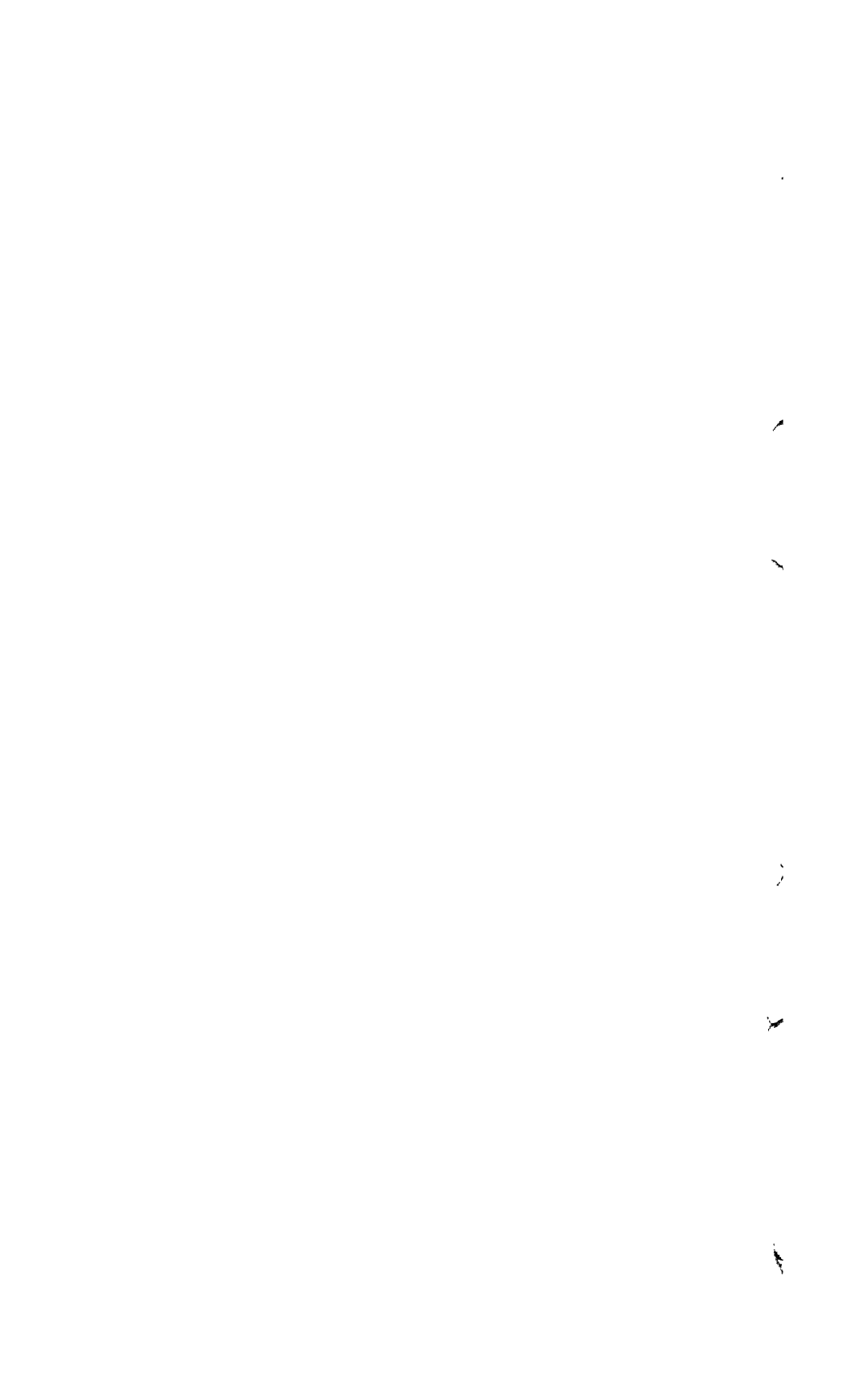
Classes 6 and 7 trees are usually too small to be merchantable. Class 6 trees grow fairly well and give promise of later development if released. They bear practically no seed. When merchantable they should be cut, unless relief from competition is insured by removal of other trees. Class 7 trees are undesirable from every standpoint and should always be cut if merchantable.

Seed trees should be of classes 1 or 3, and from 20 to 30 inches in diameter.

#### LITERATURE CITED

- (1) DUNNING, D.  
1922. RELATION OF CROWN SIZE AND CHARACTER TO RATE OF GROWTH AND RESPONSE TO CUTTING IN WESTERN YELLOW PINE. *Jour. Forestry* 20: 379-389.
- (2) ———  
1923. SOME RESULTS OF CUTTING IN THE SIERRA FORESTS OF CALIFORNIA. U. S. Dept. Agr. Bul. 1176, 27 p., illus.
- (3) HANZLIK, E. J.  
1924. TREE CLASSIFICATION IN SWEDEN. *Jour. Forestry* 22: 175-177, illus.
- (4) HECK, C. R.  
1904. FREIE DURCHFÖRSTUNG. 115 p., illus. Berlin, Julius Springer.
- (5) JAPING.  
1911. ÜBER DAS WACHSTUM DER KRAFTSCHEN STAMMKLASSEN IM VERLAUF EINER ZEHNJÄHRIGEN ZUWACHSPERIODE. *Ztschr. Forst u. Jagdw.* 43: [663]-686, illus.
- (6) KRAFT, G.  
1884. BEITRÄGE ZUR LEHRE VON DEN DURCHFÖRSTUNGEN, SCHLAGSTELLUNGEN UND LICHTUNGSHIEBEN. 147 p., illus. Hannover, Klingworth.
- (7) KRAUCH, H.  
1926. THE DETERMINATION OF INCREMENT IN CUT-OVER STANDS OF WESTERN YELLOW PINE IN ARIZONA. *Jour. Agr. Research* 32: 501-541, illus.

- (8) LAKARI, O. J.  
1920. TUTKIMUKSIA MÄNNYN MUODOSTA. (UNTERSUCHUNGEN ÜBER DIE FORM DER KIEFER.) Commun. Inst. Quaest. Forest. Finland. 3 [1], 30 p. [German résumé, 8 p. Separately paged.]
- (9) LOREY, T.  
1925. HANDBUCH DER FORSTWISSENSCHAFT. Aufl. 4, verb. u. erweit., herausgegeben von H. Weber. Bd. 2, illus. Tübingen, H. Laupp.
- (10) PEARSON, G. A.  
1923. NATURAL REPRODUCTION OF WESTERN YELLOW PINE IN THE SOUTH-WEST. U. S. Dept. Agr. Bul. 1105, 144 p., illus.
- (11) PRICE, J. H.  
1924. RELATION OF QUALITY OF LUMBER PRODUCED TO PERCENTAGE OF STAND CUT. Timberman 25 (7): 148-149, illus.
- (12) SCHOTTE, G.  
1912. OM GALLRINGSFÖRSÖK. (ÜBER DURCHFÖRSTUNGSVERSUCHE). Meddel. Statens Skogsförsöksanst. [Sweden] 9: 211-269, illus. [German summary, p. [XXXI]-XXXVIII.]
- (13) SHOW, S. B.  
1926. TIMBER GROWING AND LOGGING PRACTICE IN THE CALIFORNIA PINE REGION. U. S. Dept. Agr. Bul. 1402, 76 p., illus.
- (14) SOBOLEFF, A.  
1909. [DIE VERTEILUNG DES SAMENERTRAGES BEI DER FICHTE NACH DEN BÄUMEN DER KRAFTSCHEN STAMMKLASSEN.] (Abstract) Centbl. Gesam. Forstw. 35: 137-139.
- (15) ZON, R.  
1915. SEED PRODUCTION OF WESTERN WHITE PINE. U. S. Dept. Agr. Bul. 210, 15 p.





# AN ACANTHOCEPHALID, *PLAGIORHYNCHUS FORMOSUS*, FROM THE CHICKEN AND THE ROBIN<sup>1</sup>

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## INTRODUCTION

Some acanthocephalids from the small intestine of a chicken, collected at Vineland, N. J., and through the courtesy of J. J. Black sent to the Zoological Division of the Bureau of Animal Industry, were identified as *Plagiorhynchus formosus* Van Cleave, 1918.<sup>2</sup> This report is of interest since, so far as the writer can ascertain, no members of the Acanthocephala have been recorded from the chicken. *P. formosus* is reported here also from another new host, the robin (*Planesticus migratorius*).

## ECONOMIC IMPORTANCE

The possibility should be kept in mind that what is at first an accidental and economically unimportant transfer of a parasite of wild birds to poultry, in time may result in the parasite's adaptation to the new host and its subsequent widespread distribution. Something of this sort appears to have happened in the case of such economically important parasites as *Thysanosoma actinioides*, *Fasciola magna*, *Oesophagostomum columbianum*, and other worms. It is of interest to note that since sending in the first specimens, Black has reported finding another such acanthocephalid in a Barred Plymouth Rock cockerel.

## DESCRIPTION OF SPECIMENS

The material from the chicken consisted of one male and two females, all immature. In view of this immaturity of the specimens they were considered in some detail and compared with the type material as far as possible.

**Male.**—The body (fig. 1), which is without spines, is elliptical in shape; its maximum length is 5.5 mm. and its maximum diameter 1.02 mm. The cylindrical proboscis, bent at approximately a 60° angle with the body, is 0.725 mm. long and 0.325 mm. in greatest diameter, and is armed with 16 parallel, alternating rows of hooks with from 12 to 13 hooks in each row; the tip of the proboscis is slightly inverted. Beginning at the posterior end of the proboscis there is the following variation in hook lengths, in microns, for the 13 successive hooks in one longitudinal row: 40, 40, 56, 60, 64, 60, 60, 64, 60, 52, 56, 48, and 40. The double-

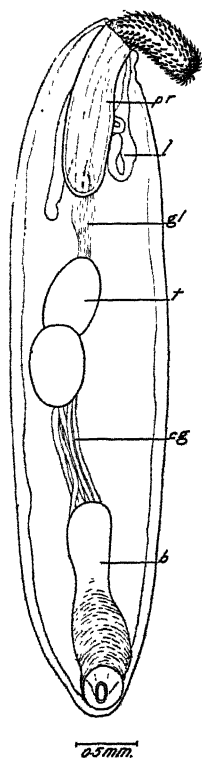


FIG. 1.—*Plagiorhynchus formosus*. Immature male from chicken: b, bursa; cg, cement glands; gl, genital ligament; l, lemniscus; pr, proboscis receptacle; t, testis

<sup>1</sup> Received for publication Mar. 22, 1928; issued July, 1928.

<sup>2</sup> VAN CLEAVE, H. J. THE ACANTHOCEPHALA OF NORTH AMERICAN BIRDS. Trans. Amer. Micros. Soc. 37: 19]-47, illus. 1918.

walled proboscis receptacle, attached to the base of the proboscis, is cylindrical with a rounded base, and measures 1.25 by 0.325 mm. The prominent lemnisci are 2.63 by 0.155 mm. in size. The testes are median in the body, and lie in tandem touching each other; they are of equal size, measuring 0.55 mm. long by 0.457 mm. broad. The six cement glands are long and slender, lie very close together, and extend from the posterior testis to the bursa.

*Female*.—The females (fig. 2) are ellipsoidal in shape and are shorter than the males. They measure 4.48 by 1.12 mm. and 3.78 by 1.28 mm. in maximum lengths and diameters. The probosces are 0.64 by 0.25 mm. and 0.78 by 0.306 mm., respectively, their tips being considerably inverted. The probosces bear 16 parallel longitudinal rows of hooks; 10 to 12 hooks in a row are to be seen clearly and 2 to 4 more per row can be made out in certain parts of the inverted tips. Beginning at the posterior ends of the probosces there are the following variations in hook lengths in microns for 12 successive hooks in a row: 40, 60, 76, 72, 68, 68, 68, 76, 78, 72, 72, and 60, in one specimen, and 48, 48, 64, 68, 72, 80, 76, 76, 76, 80, 80, and 72 in the other. The proboscis receptacles with their rounded bases measure 1.54 by 0.309 mm. and 1.54 by 0.344 mm. in the two specimens. The ovarian masses, situated medianly, are loosely lobate.

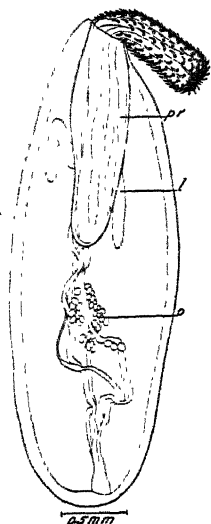


FIG. 2.—*Plagiorhynchus formosus*. Immature female from chicken: l, lemniscus; o, ovary; p, proboscis receptacle

These specimens evidently belong to the genus *Plagiorhynchus* Lühe, 1911, since they possess the following diagnostic characters of that genus: Proboscis with hooks in parallel, alternating rows; body proper without spines; no subcuticular nuclei; no spherical enlargement followed by narrow, elongated neck behind proboscis; proboscis receptacle a double-walled sac attached at base of proboscis; six cement glands present.

Van Cleave (1918)<sup>3</sup> gives this specific description for *Plagiorhynchus formosus*:

Body about 10 mm. long, elliptical to slightly ovoid. Proboscis practically cylindrical, diameter about one-third of length; armed with 16 longitudinal rows of 13 to 14 hooks each. Cement glands long, tubular. Hard-shelled embryos inside body of female elliptical, 48 $\mu$  to 60 $\mu$  by 12 $\mu$  to 20 $\mu$  in diameter.

#### COMPARISON WITH TYPE MATERIAL

The specimens from the chicken differ in certain respects from the type material, particularly in the matter of size. The type male is 8.5 by 2 mm., while the male described here is but 5.5 by 1.02 mm.; the type female is 9.5 by 2 mm., as compared to 4.48 by 1.12 mm. and 3.78 by 1.28 for the females from the chicken. The probosces show somewhat less marked differences in size, particularly if it is kept in mind that the tips of the smaller specimens are much more inverted. The types measure 1.06 by 0.33 mm., while the others are 0.725 by 0.325 mm., 0.64 by 0.28 mm., and 0.78 by 0.306 mm. The hooks also show some differences in length; those of the type material vary from above, vary from 48 $\mu$  to 80 $\mu$ , 40 $\mu$  to 78 $\mu$ , and 40 $\mu$  to 63 $\mu$ . In all specimens 65 $\mu$  in a longitudinal row, whereas the three others, as given mens the smallest hooks are at the base of the proboscs, and the longest hooks approximately halfway toward the tip.

<sup>3</sup>VAN CLEAVE, H. J. Op. cit.

These quite consistent differences in size do not seem of specific significance in view of the immaturity of the material from the chicken and also its presence in an abnormal host. Since 13 hooks are observed in most of the longitudinal rows in the proboscis of the male from the chicken, and 2 to 4 hooks are made out in the inverted tip of the female proboscis in addition to the 10 to 12 clearly seen, the possibility of a difference in number of hooks per longitudinal row from the number in the type seems negligible. A distinguishing feature, the occurrence of the testes in the new material midway in the body, rather than pre-equatorial and close to the proboscis receptacle as in the type male, is regarded as probably not of specific importance considering the difference in maturity of the specimens.

The points of agreement of the material from the chicken with the specific diagnosis of the type, aside from generic characters, include the elliptical shape of the body, the cylindrical shape and armature of the proboscis, and the long, tubular, cement glands. It is believed, therefore, that the specimens described here from a chicken are *Plagiorhynchus formosus*.

This species has been reported previously from the flicker (*Colaptes auratus*) collected at Bowie, Md., and from the crow (*Corvus americanus*) collected at Washington, D. C. A new host record of particular interest here is that of the robin (*Planesticus migratorius*). Three specimens of *Plagiorhynchus formosus* were collected by Black from a robin found paralyzed on the poultry farm from which the infected chicken had come. These specimens from the robin, a female and two males, are mature and conform closely to the type material in all respects. *Gallus domesticus* is undoubtedly an accidental host. However, the record is of economic interest as affording new data in regard to the interrelationships of wild birds and poultry in connection with the transfer of parasites from one to the other.



# EFFECTS OF FASTING AND THE METHOD OF PREPARATION OF FEED UPON THE DIGESTIVE PROCESS IN DAIRY CATTLE<sup>1</sup>

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## INTRODUCTION

Studies of the process of digestion in ruminants have contributed facts of fundamental importance to the science of animal nutrition. Investigators have studied not only the chemical reactions and transformations occurring in food substances during their passage through the alimentary canal, but also the mechanical features of the process. Researches in this field have contributed much toward a solution of the feeding problems of farm livestock.

There are still many phases of the digestive process of ruminants concerning which our knowledge is not entirely clear. Armsby<sup>3</sup> states:

In the ruminant, water and liquid feeds may pass quite directly to the abomasum, although as a matter of fact, they seem to reach all four divisions of the stomach. The more bulky feeds, however, fail to pass through the esophageal canal but enter the rumen and reticulum \* \* \*. The rumen is so large that it always contains a considerable amount of material and the new feed when swallowed is more or less completely mixed with that already in the rumen by the peristaltic action of the latter, thus tending to prolong its stay. The liquid or finely comminuted portions probably pass on directly to the omasum, or manifolds, and the abomasum, but the bulk of the feed undergoes the process of rumination.

The findings of Völtz<sup>4</sup> with regard to the course of liquids in the stomachs of sheep do not agree with the statements of Armsby, but they do agree with respect to solid foods. It was found in Völtz's experiments that—

When a sheep was killed directly after drinking a large amount of water to which alcohol had been added, nearly all of the alcohol was recovered in the paunch, showing that liquids are not immediately transferred to the abomasum.

The same holds for solid foods, as is shown by mixing with the food finely divided silver and finding this in the paunch.

Amadon,<sup>5</sup> from observations made through an artificial opening into the rumen, concludes that—

Ground feed and other concentrates may pass directly into the honeycomb during the course of the meal and we have even observed a passage of ground feed occurring from the honeycomb to the manyplies and true stomach during the eating period. The condition which determines the route to be followed is that of weight, all light food entering the back part of the paunch while a portion of the heavy food passes directly into the honeycomb \* \* \*. Whole

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<sup>3</sup> ARMSBY, H. P. *THE NUTRITION OF FARM ANIMALS*. p. 81-82, illus. New York, The Macmillan Company, 1917.

<sup>4</sup> VÖLTZ, W. ÜBER DEN DIREKTEN TRANSPORT DES FUTTERS UND DES TRÄNKWASSERS DURCH DEN SCHLUCKAKT BEIM WIEDERKÄUER. *Med. Klinik* 7: 1296-1297. 1911. (Abstract in *Expt. Sta. Rec.* 29: 66, 1913.)

<sup>5</sup> AMADON, R. S. *THE OX STOMACH. SOME FACTS WHICH CATTLE OWNERS SHOULD KNOW*. N. Dak. Agr. Expt. Sta. Bul. 196, 16 p., illus. 1926.

corn and heavy metal objects such as nails therefore remain in the depths of the honeycomb. The whole corn kernels are passed on through the digestive system but there is no evidence to show that such is the case as regards the foreign bodies, nails, etc.

It might be assumed that the fullness of the stomach at the time of a meal would affect the course of the food swallowed. In order to test the correctness of this theory, some of the animals used in these experiments were fasted for several days and some were full-fed up to the time of slaughter.

The fasting experiments are of especial interest in view of the fact that Forbes<sup>6</sup> and his coworkers have recently published a number of papers reporting experiments in which their calculations are based upon the fasting katabolism of dry cows and steers. The authors state, however, that the method is still in process of development and has not yet been standardized. The following excerpt<sup>7</sup> illustrates one of the attempts of the investigators to arrive at a standard procedure for bringing animals into a condition of true fasting.

The experimental routine in the last two experiments mentioned was characterized by one noteworthy innovation—the introduction of the idea of special treatment of the experimental subject to bring about as promptly as possible a condition of true and complete fast.

For this purpose a physic was given to steer No. 260, after which roughage was withheld for one day and grain for one-half day; then the paunch was washed out by means of a stomach pump, and an enema was given. The withholding of roughage for one day seems to have permitted the paunch to clear itself, inasmuch as it appeared to be empty when the pump was used.

### OBJECTS OF EXPERIMENTS

The experiments reported in this paper were undertaken to study the effects of (1) fasting and (2) the method of preparation of feed upon the digestive process in dairy cattle, with particular reference to the course of the feed through the stomach.

### METHODS OF PROCEDURE

The animals used in these studies were, with one exception, reactors to the tuberculin test. Careful examination of the carcasses by competent veterinarians revealed only very small tubercular lesions; so it is believed that the extent of the tubercular infection was not great enough to cause any abnormal conditions so far as these particular experiments are concerned. The one nonreactor, No. 12313, was a nonbreeder. Details regarding breed, age, and weight are given in Table 1.

Two groups of the animals were fed during preliminary periods of 10 and 14 days, respectively, and the others for as long as conditions permitted. (Table 2.) Three of the cows, Nos. 1, 11, and 742, were milking quite liberally and were therefore fed silage, hay, and a grain mixture, but the others were fed either whole or finely ground alfalfa

<sup>6</sup> FORBES, E. B., BRAMAN, W. W., KRISS, M., FRIES, J. A., COCHRANE, D. C., JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., and MAUCHER, J. V., JR. THE INFLUENCE OF THE ENVIRONMENTAL TEMPERATURE ON THE HEAT PRODUCTION OF CATTLE. *Jour. Agr. Research* 33: 579-589. 1926.

— FRIES, J. A., BRAMAN, W. W., and KRISS, M. THE RELATIVE UTILIZATION OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE, AND MILK PRODUCTION OF CATTLE. *Jour. Agr. Research* 33: 483-492. 1926.

— KRISS, M., and BRAMAN, W. W. THE COMPUTED AS COMPARED WITH THE DIRECTLY OBSERVED FASTING KATABOLISM OF CATTLE AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF ENERGY. *Jour. Agr. Research* 34: 167-179. 1927.

<sup>7</sup> FORBES, E. B., KRISS, M., and BRAMAN, W. W. *Op. cit.* p. 176.

hay as the sole feed. One group of animals was fasted from 4 to 6 days, and then, with the exception of two which were kept as controls, was given feed prepared in various ways shortly before being killed. The other group of animals was not fasted. The time which elapsed between the time of feeding and killing varied considerably for several reasons.

TABLE 1.—*Breed, age, and weight of experimental animals*

Animal No	Breed <sup>a</sup>	Age at time of slaughter	Live weight just before slaughter	How weight was determined
		Yrs. Mos.	Pounds	
1.....	Crossbred.....	3 1	900	Estimated by inspection.
11.....	Grade Holstein.....	5 6	1,200	Do.
19.....	do.....	5 6	1,175	Do.
21.....	Crossbred.....	3 5	1,000	Estimated by carcass weight
26.....	do.....	2 0	900	Do.
27.....	do.....	2 2	800	Do.
644.....	Crossbred F.....	4 7	1,000	Estimated by inspection.
718.....	Crossbred F.....	7 6	1,250	Do.
742.....	do.....	4 3	770	Scales
Guernsey bull.....	Purebred Guernsey.....	2 1	1,000	Estimated by carcass weight.
Holstein bull.....	Purebred Holstein.....	5 2	2,000	Do.
663.....	Crossbred F.....	11 7	1,260	Scales.
738.....	Crossbred F.....	4 7	1,050	Do.
12813.....	Crossbred.....	3 9	1,275	Do.

<sup>a</sup>Animals designated "crossbred" were hybrid animals descended from purebred Holsteins and Guernseys.

TABLE 2.—*Character of feed and amount consumed by experimental animals during preliminary period and just previous to slaughter*

## FASTED ANIMALS

Animal No.	Preliminary period				Fasting period	Time elapsing between feeding and slaughter	Feed consumed just previous to slaughter	
	Number of days fed	Feed consumed daily					Amount and kind	Dry-matter content <sup>a</sup>
		Silage	Hay	Grain				
		Pounds	Pounds	Pounds	Days	Hours		Kgm.
1.....	10	24	<sup>b</sup> 15	8	6	1½	3 6 pounds ground alfalfa..... 6 1 pounds ground corn..... 10 pounds whole alfalfa..... Not fed.....	3. 77 4. 00
11.....	10	30	<sup>b</sup> 18	8	5	1		
19.....	10		<sup>b</sup> 20		5			
21.....	14		<sup>c</sup> 15		4	1½	2.450 gm. dyed ground corn..... 10 pounds shelled pop corn..... 8. 5 pounds ground corn..... 6 pounds ground alfalfa..... 3. 5 pounds ground corn.....	2. 06 3. 82 3. 25 2. 34
26.....	14		<sup>c</sup> 14		4	1½		
27.....	14		<sup>c</sup> 10		4	1½		
644.....	10		<sup>b</sup> 17		5	1	4. 4 pounds shelled dent corn..... Not fed.....	2. 10 1. 68
712.....	10		<sup>b</sup> 20		6	¾		
742.....	10	( <sup>d</sup> )	<sup>b</sup> ( <sup>d</sup> )	( <sup>d</sup> )	6	2½		
Guernsey bull.....	14		<sup>c</sup> 16		4		15 pounds shelled pop corn.....	5. 73
Holstein bull.....	14		<sup>c</sup> 28		5	14		

## FULL-FED ANIMALS

663.....	4		<sup>b</sup> 26.5		0	1	About 200 gm. dyed ground corn.....	0.17
738.....	4		<sup>b</sup> 23		0	1½	8.454 gm. dyed ground corn.....	7.10
12813.....	2		<sup>b</sup> 14.6		0	6	15.1 pounds shelled dent corn.....	5.76

<sup>a</sup> Dry-matter content of feeds: Ground alfalfa, 87.71 per cent; ground corn, 84.23 per cent; whole alfalfa, 88 per cent (estimated); dyed corn, shelled corn, and pop corn, 84 per cent (estimated).

<sup>b</sup> Whole alfalfa hay.

<sup>c</sup> Finely ground alfalfa hay.

<sup>d</sup> No record of amount kept.

It was planned that all animals, with the exception of the Holstein bull, should be killed as soon as they had finished eating, but on account of the failure of some animals to eat more than a small part of the feed allotted them, the slow eating of others, and various delays encountered in the routine of the slaughtering process, the time elapsing between the commencement of the meal by the animals and the time of killing ranged from about one to six hours. Some animals required about three-fourths of an hour to complete the meal, so that part of the animals were killed within a few minutes after they had finished eating.

The general procedure followed in each instance was to slaughter the animal for meat purposes in the usual manner. After the animal had been partially raised from the floor by means of a hoist attached to the rear legs, the esophagus was severed near the stomach, and in case leakage through this opening appeared likely, it was closed by means of a wire ligature. The stomach and intestines were then removed and taken to one side.

The contents of the rumen were removed first. As the stomach lay upon the floor, a large opening was made in the upper wall of the rumen and the contents scooped into large galvanized-iron tubs by means of a rounded dish. In cases in which the rumen contents were so liquid that the liquid portion separated on standing, this liquid was dished out of the tubs and poured through a fine sieve, thus separating the liquid from the solid portions. Each portion was weighed separately. The solid portion was mixed thoroughly by hand and a sample taken by selecting handfuls at random. The liquid portion was thoroughly stirred and about 1 quart was placed in a half-gallon glass jar provided with rubber ring and glass cover.

The reticulum was next opened and the entire contents saved for analysis.

The omasum was cut away from the other compartments of the stomach, placed on a metal table, sectioned, and the contents removed as completely as possible by hand. The feed residues recovered were weighed and subsampled.

The abomasum was opened and the contents scraped out and saved for analysis.

The intestines were cut into sections and carefully pressed as they were drawn through the hand, thus removing the contents. In most cases—the exceptions being the two bulls and two of the full-fed cows—the entire intestinal contents were collected and dried for analysis.

The methods employed in collection did not yield 100 per cent of the gastrointestinal contents, since small particles adhered to the linings. It is believed that the error involved in this way is relatively small, however. Had water been employed to wash out the various parts of the tract a more nearly perfect collection could have been made, but this would have prevented the determination of the dry matter in the feed residues as they were present, which was considered a point of special interest. Further, the addition of water to the collections would have increased their bulk to such an extent that subsampling would have been necessary in most cases. Accurate subsampling of material containing both free liquid and coarse solid material is difficult. Under the plan followed, only the rumen and omasum contents and seven other samples were sub-



sampled. Another small error which it was not found possible to avoid occurred when the animals fell to the floor after being stunned or shot. In some cases 1 or 2 quarts of liquid containing a small amount of solid matter was regurgitated through the mouth and thrown out over the floor.

The samples of the liquid contents of the rumen were placed in a refrigerator. Dry matter was determined in subsamples removed by means of a pipette. The other samples and subsamples were brought to air-dry condition on the steam bath. They were carefully examined by hand and by means of a magnet, and foreign material such as nails, wire, stones, and cinders removed. With the exception of samples collected from the small intestine, the samples were then ground to pass a 1-mm. sieve and dry matter and crude fiber determined according to the methods prescribed by the Association of Official Agricultural Chemists.<sup>8</sup> It was not found possible to grind the samples of the small intestine contents because they were so sticky. Dry matter was, therefore, determined in these samples by heating the entire sample to dryness in a hot-air oven. These samples were then reduced to the necessary degree of fineness in an iron mortar and crude fiber determined in the usual manner.

#### EFFECT OF FASTING ON GASTROINTESTINAL CONTENTS

With the exception of Nos. 1, 11, and 742, which were producing milk, the cattle were fed alfalfa hay as the sole feed during the preliminary period. It was planned that this should be consumed at the rate of  $1\frac{1}{2}$  pounds daily for each 100 pounds live weight. As shown in Table 2, some of the animals were fed whole hay only, and some finely ground hay.

Considering the relatively low plane of feeding during the preliminary period and the length of the fast, surprisingly large amounts of solid and liquid matter were found in the gastrointestinal tracts of the fasted animals. (Table 3.) The rumen contents of these animals differed from those of the full-fed animals in containing a much larger proportion of liquid. Much of the liquid separated out on standing; with the full-fed animals the rumen contents showed practically no free liquid. Watering the animals shortly before slaughter seemed to have but little effect upon the amounts of liquid in the rumen. Animals Nos. 1, 11, 19, 644, and 718 were offered water on the day of the slaughter; the others were not.

The presence of these large amounts of liquid in the rumens of the fasted animals seemed to indicate that this liquid was either fulfilling the function of distending the rumen to at least part of its normal size and form, or that in the absence of sufficient feed to cause the usual muscular activity of the walls of the rumen the liquid had merely stagnated at that point. The fact that the animals drank less water during fasting than normally, and that they were very quiet and listless after the first day of fasting, gives some support to the latter view. Cow 742 was affected more severely by the fast than the other animals. The last feeding was on the morning of

<sup>8</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. Revised to July 1, 1924. Ed. 2, 335 p., illus. Washington, D. C. 1925.

March 4, at which time she was also watered. From March 5 to 9, inclusive, she drank only 34 pounds of water. During the last day or two she was unable to rise. She was killed March 10. The other animals seemed normal so far as strength was concerned, although they were much less active while fasting.

TABLE 3.—*Contents of gastrointestinal tracts of dairy cattle*

## FRESH BASIS

Animal No	Contents of—								Total recovered	Total less feed given
	Rumen			Reticulum	Omasum	Abomasum	Small intestine	Large intestine		
	Solid	Liquid	Total							
Fasted animals.	Pounds	Pounds	Pounds	Gm.	Gm.	Gm	Gm	Gm	Kgm.	Kgm.
1.....	87.5	88.5	176 0	( <sup>a</sup> )	5,561	3,176	4,416	4,320	97.5	93.1
11.....	94 0	52 0	146 0	1,379	6,909	1,642	3,787	4,535	84.5	80.0
19.....	36 0	49 0	85 0	458	6,367	1,230	4,279	( <sup>b</sup> )	57.0	57 0
21.....	40 0	19.0	59.0	569	4,131	657	2,799	2,271	37.2	34.7
26.....	70.5	27.5	78.0	( <sup>a</sup> )	4,347	590	1,861	1,876	44.1	39.6
27.....	38.0	25 0	63.0	1,272	2,974	( <sup>b</sup> )	2,235	1,645	37.7	33.8
644.....	45 0	58.5	103.5	91	4,705	2,085	5,685	3,341	62.9	60.2
718.....	30 0	9 5	39.5	160	4,947	1,331	2,583	3,066	30.0	27.5
742.....	32.0	10.5	42.5	111	6,615	2,285	2,095	5,252	35 7	33.7
Guernsey bull.....	53.5	28 0	81.5	316	6,859	1,466	6,108	7,532	59.3	59 3
Holstein bull.....	40 0	102.0	142.0	486	5,763	3,964	6,820	5,853	87.4	80.6
Full-fed animals:										
663.....	103 0	0	103 0	191	10,035	4,334	5,580	9,192	76.1	75.9
738.....	109.5	0	109.5	2,828	8,395	3,338	5,255	4,134	73.7	65.2
12313.....	106.0	0	106 0	654	8,078	1,220	3,470	2,519	64.1	57.2

## DRY-MATTER BASIS

Fasted animals										
1.....	13.4	1.9	15.3	997	265	226	340	8.77	5.00	
11.....	12.7	1 3	14 0	232	1,473	236	255	298	8.85	4.85
19.....	5.4	.6	6 0	113	1,263	156	274	557	5.08	5.08
21.....	7 2	.4	7 6	135	696	49	160	297	4.78	2.72
26.....	12.2	.4	12.6	925	77	119	264	7.10	3.28	
27.....	7.9	1 0	8.9	236	638	118	164	186	5.38	2.13
644.....	5.6	1.1	6 7	18	845	261	316	340	4.82	2.43
718.....	7.3	.3	7 6	47	880	136	148	256	4.91	2.81
742.....	6.3	.2	6 5	19	1,199	319	136	839	5.46	3.78
Guernsey bull.....	7.4	.4	7 7	62	1,205	186	410	870	6.28	6.28
Holstein bull.....	15.9	1.1	17.0	214	987	582	361	412	10.28	4.55
Full-fed animals										
663.....	12.6	0	12 6	94	2,175	533	408	1,120	10.05	9.85
738.....	24 0	0	24 0	700	2,184	408	472	407	15.08	7.97
12313.....	21.3	0	21.3	303	2,167	236	317	401	13.10	7.32

<sup>a</sup> Combined with rumen contents through error.<sup>b</sup> Fresh weights lost.

Large amounts of solid matter were also present in the rumens of the fasted animals. Masses of bulky feed weighing 36 pounds and 53.5 pounds, respectively, were recovered from the stomachs of animal No. 19 and the Guernsey bull. These animals had been fasted five and four days, respectively, and were not fed just previous to slaughter, as was the case with the other animals. The total dry matter in the rumens of these animals was 6 pounds and 7.7 pounds, respectively.

Examination of the other parts of the gastrointestinal tracts of the fasted animals revealed the presence of food material resembling in all respects that in the full-fed animals, except that in the fasted animals the amounts, upon the whole, were not so great.

The tracts of the two animals not fed after their fast, No. 19 and the Guernsey bull, contained slightly larger amounts of dry matter

than the computed amounts present in the tracts of the other fasted animals after deduction of the amounts of dry matter in the feed given just previous to slaughter. The total dry matter recovered from the gastrointestinal tracts of the fasted animals, less that in the feed consumed just before slaughter, ranged roughly from one-fourth to two-thirds of that recovered from the full-fed animals. Eighty-five per cent as much dry matter was recovered from the tract of the Guernsey bull, fasted four days and weighing about 1,000 pounds, as from No. 12313, full-fed and weighing 1,275 pounds.

TABLE 4.—*Percentage of dry matter in contents of gastrointestinal tracts of dairy cattle*

Animal No.	Percentage of dry matter in contents of—					
	Rumen	Reticu- lum	Omasum	Aboma- sum	Small in- testine	Large in- testine
1.....	8.69		17.93	8.35	5.12	7.85
11.....	9.59	16.84	21.35	14.36	6.73	6.57
19.....	7.09	24.57	19.85	12.72	6.40	
21.....	12.88	23.08	16.85	7.48	5.72	13.08
26.....	16.15		21.29	13.07	6.32	14.07
27.....	14.13	18.55	21.43		7.32	11.93
644.....	6.47	19.73	17.96	12.51	5.36	10.13
718.....	19.24	29.07	17.79	10.22	5.73	8.35
742.....	15.29	17.44	18.12	13.95	6.49	15.98
Guernsey bull.....	9.57	19.72	17.57	12.66	6.72	11.55
Holstein bull.....	11.97	44.03	17.13	14.67	5.29	7.04

FULL-FED ANIMALS						
663.....	12.23	49.23	21.67	12.30	7.32	12.18
738.....	21.92	24.74	25.01	12.21	8.99	9.84
12313.....	20.09	46.37	26.83	19.36	9.14	15.93

TABLE 5.—*Percentage of total contents of gastrointestinal tracts found in each division (dry-matter basis)*

Animal No.	Percentage of total gastrointestinal content in—					
	Rumen	Reticu- lum	Omasum	Aboma- sum	Small in- testine	Large in- testine
1.....	79.2		11.4	3.0	2.6	3.9
11.....	71.9	2.6	16.7	2.7	2.9	3.4
19.....	53.7	2.2	24.9	3.1	5.4	11.0
21.....	72.2	2.8	14.6	1.0	3.3	6.2
26.....	80.6		13.0	1.1	1.7	3.7
27.....	75.1	4.4	11.9	2.2	3.0	3.5
644.....	63.1	.4	17.5	5.4	6.6	7.1
718.....	70.3	1.0	17.9	2.8	3.0	5.2
742.....	54.0	.3	22.0	5.8	2.5	15.4
Guernsey bull.....	56.4	1.0	19.2	3.0	6.5	14.0
Holstein bull.....	73.2	2.1	9.6	5.7	3.5	4.0

FULL-FED ANIMALS						
663.....	56.9	0.9	21.6	5.3	4.1	11.1
738.....	72.3	4.6	14.5	2.7	3.1	2.7
12313.....	73.9	2.3	16.5	1.8	2.4	3.1

A notable feature of the recoveries was that the digestive processes seemed to be proceeding in a normal manner in the omasums, abomasums, and small intestines of the fasted animals, as judged by the percentage of dry matter in the contents of these organs (Table 4), and by the proportion of the total content of the gastrointestinal tracts found in each division (Table 5). While there was considerable variation in the percentages of dry matter in the contents of the different divisions, the results are consistent in showing a close resemblance between the fasted and full-fed animals. It is interesting to note in this connection the relatively high dry-matter percentage in the reticulum contents, indicating that, as recovered in the usual methods of slaughter, this compartment of the stomach is not a "water bag." Further, with but one exception, the omasum contents had higher dry-matter percentages than the contents of the other divisions of the tract except the reticulum. The percentage of dry matter in the reticulum contents would fall below that of the omasum contents in several other instances if computed after deduction of the foreign matter (nails, wire, etc.). The small intestine contents were quite liquid in consistency, the percentage of dry matter in all cases being less than 10 per cent. It is possible that the rather high dry-matter percentage in the large intestine contents of the fasted animals was, in part, due to stagnation of the feed residues. It was noted at the time of recovery that this apparently had occurred in cow 742, an animal which was inactive during her last few days.

Further support for the theory that digestion was proceeding in a fairly normal manner in the fasted animals is shown in Table 5. The proportion of the contents of the total gastrointestinal tracts found in the different divisions of the control animals, No. 19 and the Guernsey bull, were not greatly different from those of the other animals, both fasted and full-fed, and indicated that each division contained about its normal share of feed or feed residues.

The feces of three of the animals were collected during the fasting period, the collections beginning about 10 hours after commencement of the fast. The data in Table 6 show that there was some irregularity in excretion, possibly due in part to the close confinement of the animals in stalls, but that upon the whole, digestion, as indicated by elimination of feed residues, was proceeding at an active rate up to and including the fourth day of fasting.

TABLE 6.—Dry matter voided in feces during fasting

Animal No.	Date	Length of collection period	Fresh weight of feces	Dry matter	Dry matter voided daily
		Hours	Grams	Per cent	Grams
21.....	Nov. 9, 1926	14½	4,921	25.8	1,280
21.....	Nov. 10, 1926	24	4,108	22.0	904
21.....	Nov. 11, 1926	24	2,228	26.2	584
21.....	Nov. 12, 1926	24	2,169	27.4	594
26.....	Nov. 9, 1926	14½	2,405	21.4	515
26.....	Nov. 10, 1926	24	2,539	21.6	548
26.....	Nov. 11, 1926	24	2,465	20.8	513
26.....	Nov. 12, 1926	24	1,683	22.0	370
27.....	Nov. 9, 1926	14½	2,560	24.8	635
27.....	Nov. 10, 1926	24	4,344	17.2	747
27.....	Nov. 11, 1926	24	2,635	14.6	385
27.....	Nov. 12, 1926	24	2,404	13.8	332

## EFFECT OF METHOD OF PREPARATION OF FEED ON GASTRO-INTESTINAL CONTENTS

The alfalfa hay fed during the preliminary period and just previous to slaughter, was fourth-cutting western hay, containing about 55 per cent leaves and having a high green color. The color was so pronounced that the gastric contents of animals fed hay only were of a bright green color. This made it very easy to determine the location of the corn fed just previous to slaughter.

It was not possible to make quantitative separations of the feed consumed just before slaughter and that already in the stomach, chiefly on account of the intimate mixing of the rumen contents which it was found had taken place. Therefore, in all but one or two cases in which separations were attempted, observations were made and notes taken regarding the approximate proportions of the recently consumed feed found in the different divisions.

The animals fed whole alfalfa hay during the preliminary period retained amounts of dry matter in their rumens ranging from about 1.5 to 7 pounds. This calculation is based upon the assumption, made merely for purposes of comparison, that all of the feed consumed just before slaughter remained in the paunch. These amounts are no greater than those in the case of the animals fed the finely ground hay.

If it be assumed, as Armsby<sup>9</sup> implies, that "finely comminuted portions probably pass on directly to the omasum, or manifolds, and the abomasum," then cattle fed on finely ground hay for 14 days and subsequently fasted for 4 or 5 days—as was the case with animals 21, 26, 27, and the Guernsey and Holstein bulls—should have no alfalfa residues in the rumen. In these experiments, however, all of these cattle had considerable amounts of hay residues in their rumens. In the case of the Guernsey bull, the hay residues contained 7.7 pounds of dry matter, while in the other cases the dry-matter content ranged from about 1 to 4 pounds.

There seemed to be little difference between the animals fed whole hay and those fed ground hay, with respect to the amounts of dry matter of the rumen contents after deducting the amounts in the feed eaten just before slaughter. Furthermore, no differences in the amounts of feed which might be attributed to the method of preparation of the hay could be observed in the other parts of the alimentary canal. The ground alfalfa consumed by Nos. 1 and 644 was recovered chiefly in their rumens.

Referring again to Armsby's statement regarding the course of the finely comminuted feed, it would be inferred that if his assumption is correct, finely ground corn would not enter the paunch, or at least most of it would not. Amadon's<sup>10</sup> theory that the course of the feed is determined largely by its weight would seem to imply that a portion of a heavy feed, such as ground corn, would enter the reticulum directly.

Most of the corn fed was recovered in the rumens and reticula, except in the case of the Holstein bull. This was true whether the corn was fed as shelled corn or ground corn. Shelled pop corn was fed to two animals upon the assumption that few of these kernels

<sup>9</sup> ARMSBY, H. P. Op. cit.

<sup>10</sup> AMADON, R. S. Op. cit.

would be broken by the animals in eating, which proved to be the case. Some coarsely ground yellow corn was dyed a brilliant red color by treating it with Congo Red 4B in water at a temperature of 110°–120° F., using about 1 gm. of the dye per pound of corn. After standing about four hours, the corn was washed by decantation until the wash water was nearly clear, dried in shallow pans on the steam bath, and reground. The dye did not color perceptibly the other alimentary contents of the animals consuming the dyed corn.

It was very easy to distinguish the bright red corn mixed with the green hay residues. One of the most surprising features in connection with the recovery of the rumen contents was the thorough mixing of the newly eaten feed with the residues already in the rumen, which had occurred during the relatively short intervals between feeding and killing. The mixing had been done almost as thoroughly as could be done by hand or by means of a mechanical mixer. A slight exception to this general condition was found in the case of No. 738, fed a large amount of ground corn. Here the corn was intimately mixed with the hay residues, as in the other animals, except that 100–200 gm. of corn was found practically unmixed in the lower part of the rumen sacs. This was but a small part of the whole, however.

In the case of animals fed large amounts of ground corn, notably Nos. 27 and 738, a considerable quantity was recovered in the reticula, although this was not over one-third of that fed. A thorough mixing of the contents of this compartment had occurred also, and the corn formed a larger proportion of the feed present than in the rumen.

In but few cases was any of the feed consumed just before slaughter found in the omasum. In cow 718 some ground corn was present in the reticulum, but none in the omasum or abomasum. Animal No. 27 had a rather large quantity of corn in the reticulum but only about 1 ounce in the omasum. In No. 21 a very small amount of dyed corn was noted along the lower edges of the leaves of the omasum. In the case of cow 738, the bright red corn was mixed with the hay along the ventral side of the omasum and had spread out from the opening about one-fifth to one-fourth of the distance from the opening to the opposite side of the compartment. No corn was found in the abomasum. Cow 663 refused to eat more than part of a pound of her feed, and it was very difficult to trace this small amount accurately after it had become mixed with the large mass of hay residues present. None of the corn was noted in the omasum, however.

Ten pounds of shelled pop corn was fed to No. 26 about one and one-half hours before slaughter. No broken kernels were observed. The bulk of the corn was found in the rumen, with a considerable quantity in the reticulum. Only a few kernels (10 to 15) were noted in the omasum and none were recovered in the abomasum.

Heifer 12313 was fed 15.1 pounds of shelled corn six hours before she was killed. Most of this was recovered in the rumen. After air drying the samples of material recovered from the alimentary tract, the corn kernels were separated and weighed. Calculating the recovery in the samples upon the basis of the total contents of the different divisions, the corn recovered formed 79 per cent of that fed, 70 per cent being found in the rumen, 3 per cent in the reticulum, 4 per cent in the omasum, and 2 per cent in the abomasum. About

4 gm., or 0.1 per cent, were also found in the large intestine, but it is believed that this, as well as part of that found in the abomasum, was corn from silage fed previous to the two-day preliminary period.

The Holstein bull was fed 15 pounds of shelled pop corn the evening before slaughter. The chief solid contents of the rumen and reticulum of this animal consisted of pop corn. The small amount of other solid material present consisted chiefly of bedding material and some hay. No bedding material was recovered in the other animals. Hay residues were present in the omasum, however. As in the case of the other animals fed shelled corn, most of the corn fed this animal was recovered in the rumen, with a portion in the reticulum. In the omasum the kernels of corn were quite generally distributed through the leaves along the ventral side, but had not yet reached the dorsal portion. A few ounces of corn were recovered in the abomasum. The corn had progressed farther into the omasum than in the case of the other animals.

TABLE 7.—*Fineness of grinding of feeds given during preliminary period and just previous to slaughter*

Diameter of sieve openings	Proportion of sample retained on sieves		Diameter of sieve openings	Proportion of sample retained on sieves	
	Alfalfa hay	Shelled corn		Alfalfa hay	Shelled corn
<i>Mm.</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Mm.</i>	<i>Per cent</i>	<i>Per cent</i>
5.....	0	0	1.....	60	28
3.....	0	0	0.5.....	29	27
2.....	4	3	Bottom pan.....	7	42

The mixture of ground corn and ground hay fed to animal No. 1 was recovered chiefly in the rumen. Both these feeds were very finely ground, as shown in Table 7. All of the hay used in the experiment, including that in the preliminary period, whether fed in whole or ground form, was originally of the same lot and quite uniform in quality. The analyses of these feeds are shown in Table 8.

The muscular movements of the rumen seemed to have been much more active in the full-fed than in the fasted animals, as judged by the completeness of mixing of the feed recently eaten with that already in the rumen.

TABLE 8.—*Composition of feeds used*

Kind of feed	Dry matter	Ash	Crude protein	Crude fiber	Ether extract	Nitrogen free extract
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Ground corn.....	84.23	1.50	9.21	2.11	2.22	69.19
Ground alfalfa.....	87.71	9.58	16.64	26.37	2.30	32.82

TABLE 9.—Crude-fiber content of material removed from gastrointestinal tracts

## FRESH BASIS

Animal No.	Crude fiber in material removed from—					
	Rumen <sup>a</sup>	Recti- culum	Omasum	Aboma- sum	Small intestine	Large intestine
Fasted animals:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	4.61	-----	9.64	3.02	0.54	4.12
11.....	4.98	8.16	8.07	4.64	.15	2.76
19.....	4.29	10.32	9.79	4.78	1.17	-----
21.....	3.12	4.16	7.50	2.82	1.24	5.34
26.....	3.05	-----	9.24	2.59	.63	5.61
27.....	2.53	2.94	6.75	-----	1.29	4.22
644.....	3.67	9.43	9.87	7.19	1.54	5.63
718.....	5.72	6.40	9.13	5.02	.89	4.03
742.....	4.68	5.60	6.84	3.09	.42	4.80
Guernsey bull.....	5.52	9.95	7.34	3.97	1.52	4.51
Holstein bull.....	1.00	4.73	7.25	3.99	.61	2.84
Full-fed animals:						
663.....	5.90	7.94	8.31	4.24	.90	4.73
738.....	4.54	5.41	8.34	4.66	1.55	3.55
12313.....	5.41	4.00	8.62	3.77	1.04	3.97

## DRY-MATTER BASIS

Fasted animals:						
1.....	53.08	-----	53.76	46.91	10.63	52.28
11.....	51.92	48.46	37.82	32.33	2.17	41.99
19.....	60.76	42.01	49.35	37.56	18.35	46.53
21.....	24.24	17.57	44.53	37.65	21.74	40.81
26.....	18.91	-----	43.39	19.85	9.92	39.89
27.....	17.91	15.83	31.47	29.89	17.69	37.35
644.....	56.76	47.81	54.95	57.44	27.78	55.31
718.....	29.75	22.00	51.33	49.10	15.52	48.32
742.....	30.63	32.12	37.73	22.12	6.46	30.04
Guernsey bull.....	57.70	50.47	41.78	31.37	22.64	39.01
Holstein bull.....	8.37	10.75	42.33	27.20	11.58	40.27
Full-fed animals:						
663.....	48.26	16.13	38.37	34.47	12.23	38.81
738.....	20.72	21.87	33.99	38.15	17.22	36.06
12313.....	26.95	8.63	32.11	19.45	11.43	24.91

<sup>a</sup> The calculations in this column are based upon crude-fiber determinations of the samples of the solid portion of the rumen contents. The relatively small amounts of dry matter in the liquid portion of the rumen contents are not included.

## CRUDE FIBER IN GASTROINTESTINAL CONTENTS

The percentages of crude fiber in the gastrointestinal contents (Table 9), when calculated upon the fresh basis, furnish little or no information regarding the nature of the material, owing chiefly to differences in the percentages of dry matter. The percentages of crude fiber in the omasum contents of the different animals are upon the whole higher than those in the contents of the other divisions and show some degree of similarity, the range being roughly from 7 to 10 per cent. The crude fiber percentages in the small intestine contents are low, all being less than 2 per cent.

When calculated in terms of the percentages of the dry matter comprised by crude fiber, some interesting figures were obtained. The two highest crude-fiber contents in the rumen samples were those of cow 19 and the Guernsey bull, the animals not fed after the fasting period. These high percentages are about twice as great as the crude-fiber content of the hay fed during the preliminary period. (Table 8.) Since the feed residues recovered from these animals appeared to consist of alfalfa hay only, it is assumed that the more readily soluble



portions of the hay had been removed in the processes of digestion, so that the residues remaining in the rumen consisted largely of crude fiber. Further support for this theory is found in the data for Nos. 11 and 644, fed alfalfa hay only, which show high percentages of fiber in the rumen contents. Then, too, these crude-fiber percentages correspond roughly to those of the large intestine contents, from which presumably most of the digestible material had been absorbed.

All the animals fed corn shortly before slaughter, with the exception of Nos. 1 and 663, show distinctly lower percentages of fiber in the rumen contents (dry-matter basis) than the other animals. The presence of the corn was undoubtedly responsible for these striking differences. Cow 663 ate only 200 gm. of corn, which could have had but little effect in lowering the fiber in the rumen contents. The quantity of fiber in her case corresponds to that of the animals fed hay only. No explanation is at hand for the high percentage of fiber in the rumen of animal No. 1, fed both hay and corn.

The quantity of fiber present in the reticula of these animals corresponds in a general way to that in the rumens. The animals not fed or fed hay only have high proportions of fiber in both rumens and reticula, whereas those fed corn in considerable quantity have low percentages of fiber in the contents of both compartments.

The crude fiber in the omasum contents is high, although there is a rather wide variability. Corn was found in but few of the omasums and then in but small amounts, the contents apparently consisting chiefly of alfalfa residues. Possibly the differences in the fiber contents represent differences in the completeness of absorption of the more soluble constituents of the hay.

Considerable variability is shown in the crude-fiber percentages of the abomasum contents, although in general these are high.

Crude fiber formed a surprisingly low proportion of the contents of the small intestine. Possibly this may be accounted for by the presence of mucus, salts, and digestive juices.

The contents of the large intestine contained a large proportion of crude fiber, except in the case of No. 12313. The small percentage in this instance may have been caused by the mixed ration fed previous to the two-day preliminary period. Some kernels of corn were noted in the contents of the abomasum and large intestine.

Considerable difficulty was encountered in obtaining checks of duplicate determinations of crude fiber owing to the presence of sand and like material in the samples. The female animals had access to steamed bone meal and finely ground limestone, provided in self-feeders, and the presence of pebbles and cinders in the abomasums indicated that they may have consumed some dirt about the yards.

Of the 82 determinations of crude fiber in the air-dry material, 35 of the determinations made in duplicate checked within 0.4 per cent, 23 differed from 0.45 to 0.80 per cent, and the remainder differed from 0.85 to 1.3 per cent.

#### FOREIGN MATTER IN GASTROINTESTINAL CONTENTS

Foreign matter, such as nails, wire, glass, and stones, was found in the stomachs of all the experimental animals. The amounts recovered from each division are shown in Table 10. With the exception

of the two cases in which the reticulum contents were included with the rumen contents through error, foreign substances were present in all reticula and abomasums and in a few cases in the rumens. No



FIG. 1.—Foreign matter removed from the reticulum of animal No. 11. The nature of this material is typical of that recovered from the reticula of the animals in these experiments

foreign bodies of perceptible size were found in the omasums, small intestines, or large intestines, although considerable sand or similar material was present in some of these. The foreign matter found in the reticula consisted chiefly of nails, wire, glass, and sand (fig. 1), while in the abomasums it was largely small pebbles and cinders (figs. 2, 3, and 4). These findings indicate a movement of at least a part of the foreign material through the stomach. The statement of Amadon<sup>11</sup> that "whole corn kernels are passed on through the digestive system, but there is no

evidence to show that such is the case as regards the foreign bodies, nails, etc.," is not therefore wholly in agreement with the results here shown.

TABLE 10.—Amounts of foreign matter removed from gastrointestinal tracts of dairy cattle

Animal No.	Foreign matter removed from—					
	Rumen <sup>a</sup>	Reticulum	Omasum	Abomasum	Small intestine	Large intestine
	Grams	Grams	Grams	Grams	Grams	Grams
1.....	7	0	0	16	0	0
11.....	0	25	0	51	0	0
19.....	5	40	0	40	0	0
21.....	0	10	0	2	0	0
26.....	0	0	0	2	0	0
27.....	1	6	0	15	0	0
644.....	0	9	0	3	0	0
718.....	7	2	0	3	0	0
742.....	0	2	0	13	0	0
Guernsey bull.....	0	19	0	7	0	0
Holstein bull.....	3	30	0	52	0	0
663.....	0	60	0	24	0	0
738.....	0	10	0	31	0	0
12313.....	0	27	0	15	0	0

<sup>a</sup> Figures show the amount of foreign matter recovered from subsamples. The dry matter of the subsamples formed but a small proportion of the total dry-matter content of the rumen; in the case of No. 1, the percentage being 8.6; for No. 19, 12.1 per cent; No. 27, 13.7 per cent; No. 718, 11.9 per cent; and for the Holstein bull, 10.5 per cent.

<sup>b</sup> May have come from reticulum, since reticulum contents were combined with those of rumen through error.

<sup>11</sup> AMADON, R. S. Op. cit.

## THE RUMEN AS A STOREHOUSE

Statistical constants were derived from the data of Table 5, with the object of studying the variability of the data showing the proportion of the total dry matter of the gastrointestinal contents found in each division of the tract. It is shown in Table 11 that these data, upon the whole, have a high degree of variability. This is to be expected on account of the differences in the amounts and character of the feed ingested. Even with these wide differences in feed intake,

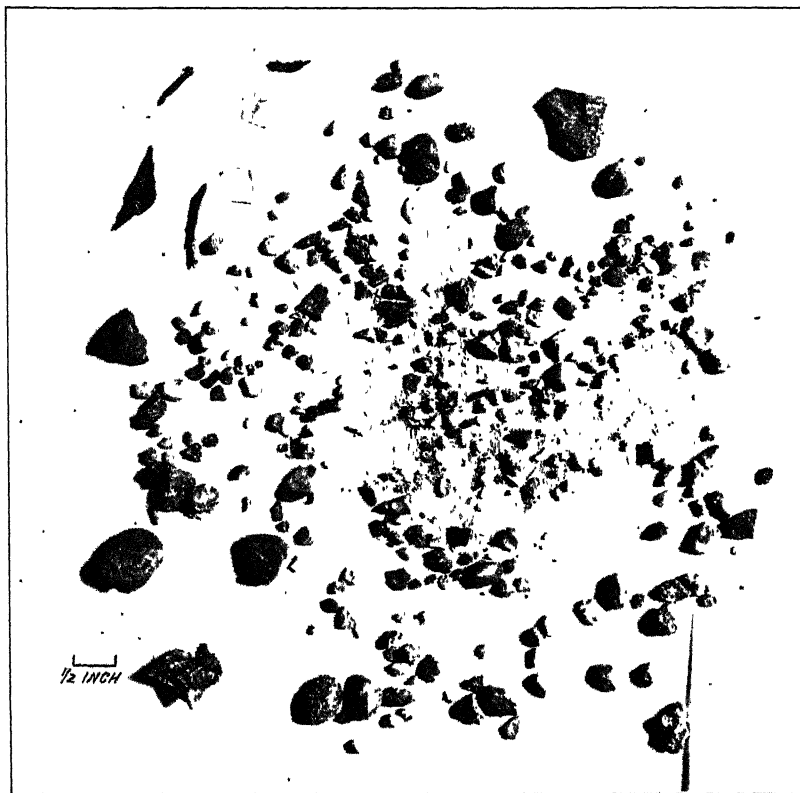


FIG. 2.—Foreign matter recovered from the abomasum of animal No. 11. This matter, which consisted chiefly of stones and cinders, was typical of that secured from the abomasums of all the animals. In this particular instance pieces of glass and metal were present also

however, it is remarkable that the variability of the fasted group so closely corresponds to the variability of all animals taken as a group.

The data for the rumen contents show a smaller variability than those for any of the other divisions. The statistical constants for this compartment indicate that the rumen retains a rather constant proportion of the total dry matter of the gastrointestinal contents. One function of the rumen, therefore, seems to be to act as a storehouse, helping to regulate the rate of passage of food to the other compartments of the stomach.

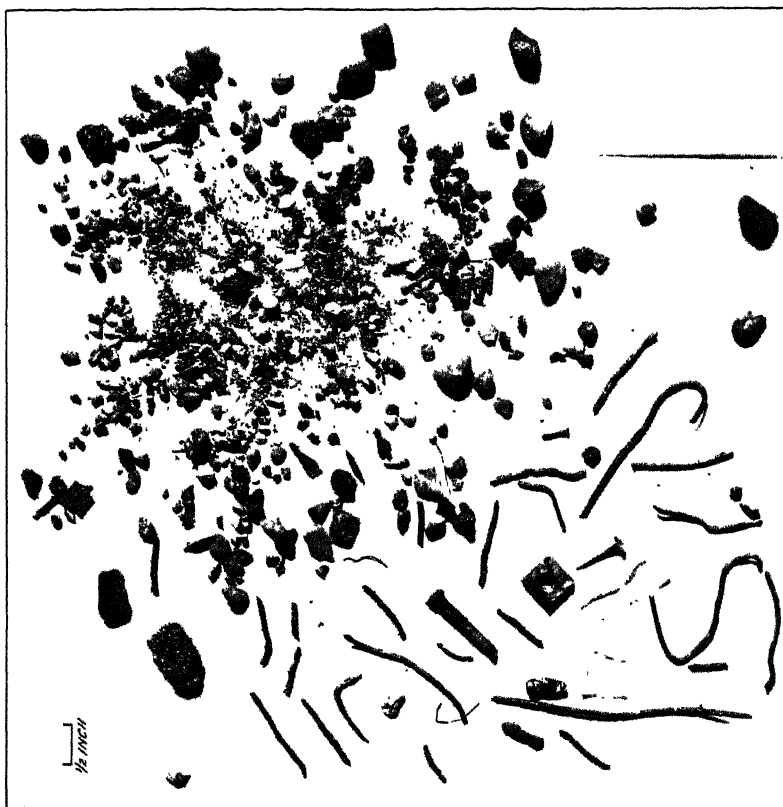


FIG. 3.—Foreign bodies separated from the gastric contents of cow No. 19. Besides numerous small stones and cinders, pieces of wire and glass, a tack, a burr, and a bullet were found.

TABLE 11.—Statistical constants of the proportion of total contents of gastrointestinal tracts found in each division (from Table 5)

FASTED ANIMALS

Division of tract	Statistical constants		
	Mean	Standard deviation	Coefficient of variability
Rumen.....	68.54±0.019	9.54±0.014	13.92±0.394
Reticulum.....	1.80±.003	1.24±.002	68.89±1.072
Omasum.....	16.05±.010	4.68±.001	29.16±.173
Abomasum.....	3.20±.0003	1.62±.0002	50.63±.5227
Small intestine.....	3.66±.0003	1.59±.0002	43.44±.381
Large intestine.....	7.02±.009	4.26±.006	60.68±.747

ALL ANIMALS

Rumen.....	68.28±1.671	9.26±1.181	13.56±0.332
Reticulum.....	2.01±.263	1.35±.186	67.16±.879
Omasum.....	16.32±.783	4.34±.554	26.59±1.275
Abomasum.....	3.22±.286	1.59±.202	49.38±.440
Small intestine.....	3.54±.205	1.45±.187	40.96±3.110
Large intestine.....	6.70±.767	4.26±.542	63.58±7.295

## SUMMARY AND CONCLUSIONS

Dairy cattle 2 years old or over were fasted from four to six days. The gastrointestinal tracts of these animals contained from one-fourth to two-thirds as much dry matter as those of animals full-fed up to the time of slaughter. Large amounts of free liquid were present in the rumens of the fasted animals, whereas little or none was present in the rumens of the unfasted cattle. Digestion seemed to have been proceeding in a normal manner (except for quantity) in the fasted animals, as judged by the percentages of dry matter



FIG. 4.—Materials found in the stomach of the Holstein bull. In this and other cases, some of the pieces of metal appeared to have been flattened, as if by the buhrs of a feed grinder. The heavy nail shown in the lower part of the illustration was found in the rumen.

and the proportions of the total contents found in each division of the gastrointestinal tract and by the output of dry matter in the feces.

Three animals were fed only whole alfalfa hay during a 10-day preliminary period previous to fasting, while five animals were fed only finely ground alfalfa hay for 14 days previous to fasting. The form in which the hay was fed seemed to have no effect upon the amount of dry matter in the rumens.

The larger part of both finely ground and shelled corn fed to different animals shortly before slaughter was recovered in the rumens,

where it had been thoroughly mixed with the other material present. A small portion of the corn was found in the reticula, while in a few cases some corn was found in the omasums. The method of preparation of the corn seemed to bear no relation to the path it followed through the stomach. The mixing of ground corn and ground hay before feeding (one animal only) seemed to yield no different results than when these feeds were given separately.

Crude fiber formed a high percentage of the dry matter in the stomach contents of the animals fed alfalfa only, while it formed a distinctly lower proportion in the rumen and reticulum contents of most of the animals which were fed corn shortly before being killed. On account of the high percentages of crude fiber in the rumen contents of the animals fed alfalfa only and then fasted, which correspond roughly to the crude-fiber contents of the reticulum, omasum, and large intestine, the theory is advanced that in these cases removal of the more digestible and soluble portions of the alfalfa took place in the rumen. Insufficient data are at hand to show whether a similar action occurred in the full-fed animals.

Foreign matter was found in the reticula and abomasums of all animals in which the contents of these divisions were recovered, indicating a movement of a part of such material at least through the digestive tract.

A function of the rumen seems to be that of a storehouse which, as judged by the coefficient of variability, retains a fairly constant proportion of the dry matter of the total gastrointestinal contents, even with a highly variable feed intake.

# THE RELATION OF ENVIRONMENT TO SHAPE OF FRUIT IN CUCUMIS SATIVUS L. AND ITS BEARING ON THE GENETIC POTENTIALITIES OF THE PLANTS<sup>1</sup>

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## INTRODUCTION

Genetic data on size and shape of fruit in *Cucumis sativus* L. are subject to much criticism unless corrected for the influence of environment and plant vigor on the development of the fruit. The difficulty of securing reliable data on shape and size of fruit in cucumber-inheritance studies suggested a number of experiments to determine what effect time of pollination, amount of pollen, soil nutrients, light, and maturing fruits would have on the final shape of newly pollinated flowers.

Commercial growers are very much concerned over the production of large proportions of "seconds" and "nubbins" under various conditions of environment. It is a common phenomenon that a variety produces different shaped fruits under different conditions of environment, and often a variety is condemned for producing poorly shaped fruits when in reality the shape was determined by conditions which have nothing to do with the genetic possibilities of the variety.

Many types of fruit shapes have been isolated through self-fertilization. The establishment of apparently homozygous strains for shape of fruit shows that certain types may be represented as definitely genetic entities. Such shapes which have been definitely fixed may be characterized as wasp, long necks, square shoulders, pointed tips, and those more or less wedge-shaped or cylindrical.

In Figure 1, A, the fruits in the top row represent genetic "wasps," having no ovules in the embryo sacs of the basal portion of the fruit. The bottom row represents physiological wasps in which all the separate seed cavities contain either seeds or rudimentary embryos.

The hereditary types do not concern us in this paper. There are, however, a number of deviations in shape from a normal variety, genetically fixed for shape of fruit, which are discussed. Many irregular types are found on vigorous vines which genetically should produce ideal fruit.

## IMPORTANCE OF LOCATION OF PISTILLATE FLOWERS

The first set of fruit on healthy, vigorous vines is heavier than the plant can successfully mature, and only the fruits<sup>3</sup> which mature earliest represent ideal types for the variety. The late-maturing fruits may deviate in various degrees from the normal, depending on the time of pollination, interruptions in the growth cycle, and

<sup>1</sup> Received for publication Mar. 7, 1928; issued July, 1928. Published with permission of the director of the Massachusetts Agricultural Experiment Station.

<sup>2</sup> Thanks are due Prof. W. D. Whitcomb for his helpful criticisms in the preparation of this manuscript.

<sup>3</sup> The term "fruit" is used to designate the stage between fertilization and maturity.

vigor of the plant. Pistillate flowers are produced on a plant until the fruit from the first pollinated flower attains sufficient size to exert an inhibitory effect, when many flowers which have not reached anthesis dry up. If growth is inhibited after a flower is pollinated and fertilization has taken place, the ovary<sup>4</sup> may remain on the

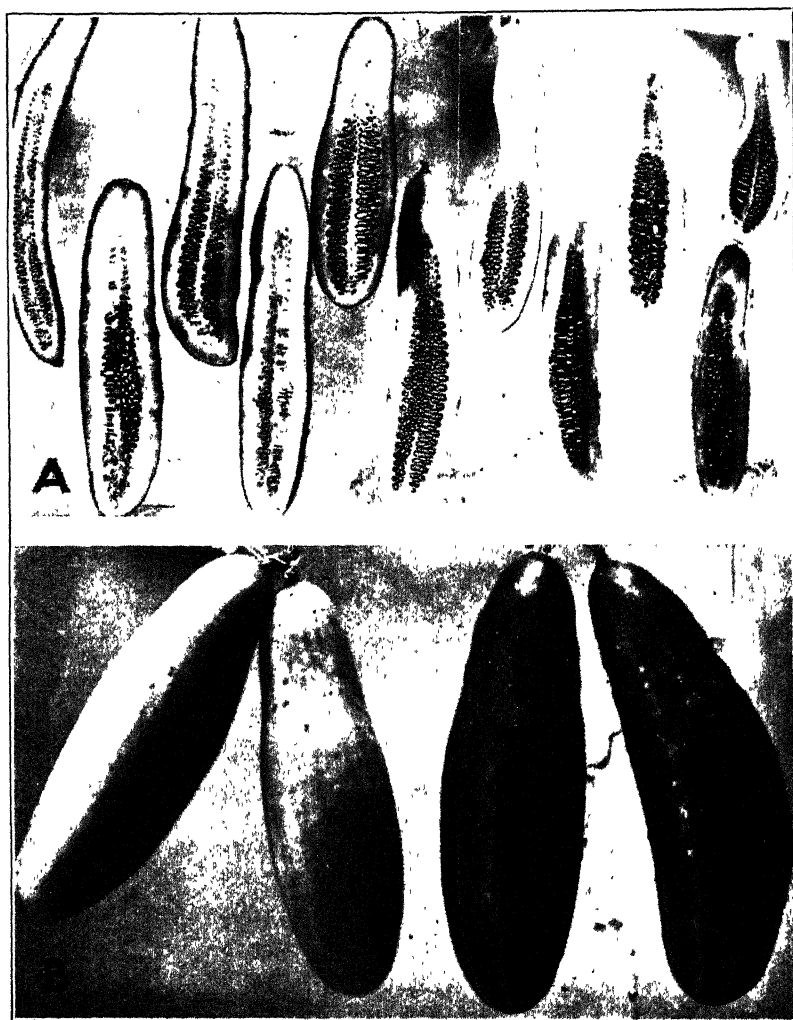


FIG. 1.—A, Cucumber fruits showing genetic “wasps” (top row) which have no ovules in the embryo sacs of the basal portion; physiological “wasps” (bottom row) the seed cavities of which contain seeds or rudimentary embryos; B, two pairs of morphological twins which developed as a result of the simultaneous pollination of two flowers produced at a single node

vine for a long period without increasing in size, but when conditions become favorable it will begin to grow. The effect of such interruptions depends on the type of plant. Some plants produce pistil-

<sup>4</sup> “Ovary” is used to designate the part of the flower that forms the cucumber from the time it is distinguishable in the bud until fertilization takes place, which is usually five or six days after anthesis.



late flowers on practically every node while others produce them only on the nodes of the laterals, and then rather sparingly. The latter type produces a greater proportion of well-shaped fruit because the set is much lighter, although the total yield may be practically the same.

If it happens that two flowers are produced at a node at one time, and are pollinated simultaneously, they develop morphological twins. Two pairs of such twins are shown in Figure 1, B. These are from

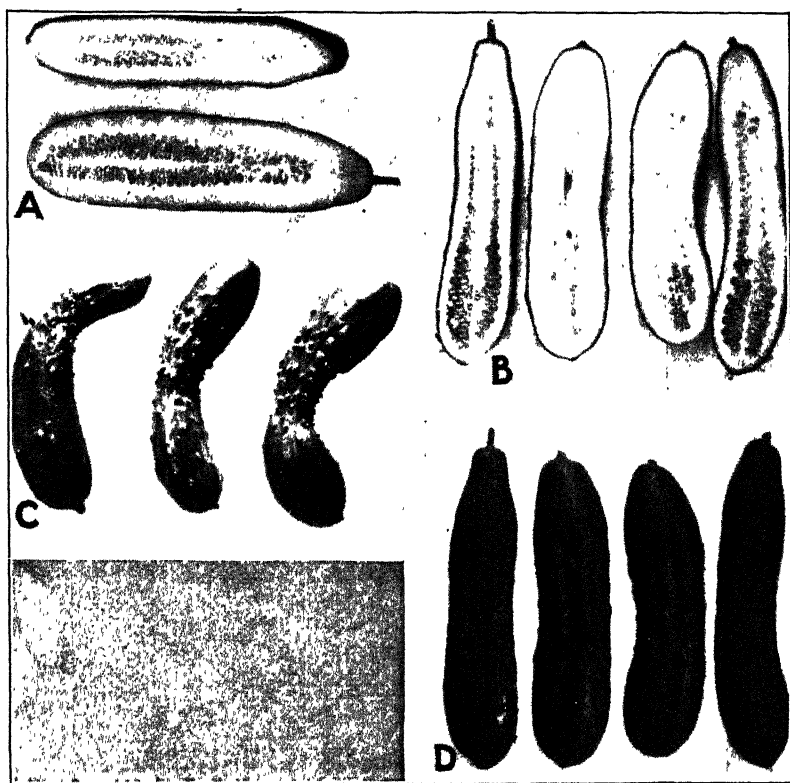


FIG. 2.—Cucumber fruits which developed from flowers pollinated 24 hours after anthesis. All are wasp-shaped. Those shown in A may be classed as first grade but not fancy; those shown in B, C, and D are seconds or culls

two varieties having well-shaped fruit of medium length. The uniformity of the twins is characteristic but their shape and size do not conform to the variety type.

#### EXPERIMENTAL DATA

##### EFFECT OF TIME OF POLLINATION ON SHAPE OF FRUIT

To obtain some definite information on the interrelation of fertility of the soil, time of pollination, and shape of fruit, two beds of 50 plants each were planted to a known variety of greenhouse cucumber. The plants were all permitted to mature a few fruits of the first set so that the available nitrogen in the soil would be materially reduced. As soon as the plants showed signs of decreasing vigor, apparently

due to a lack of nitrogen, bed No. 1 was given a copious supply of nitrate of soda; bed No. 2 received no additional nutrients. On April 16, when the nitrate of soda was applied, all the maturing fruits and fertilized ovaries were removed from the vines. All unopened flowers were covered with glassine paper bags and hand-pollinated. On 25 plants in each bed the flowers were pollinated at anthesis, while on the remaining plants the flowers were pollinated 24 hours after anthesis. At the end of 14 days the first pollinated flowers had formed fruits ready to pick for market purposes. The results may be summarized as follows:

Bed No. 1. Nitrate of soda added.

- A. Flowers pollinated at anthesis. The first 100 fruits were normal.
- B. Flowers pollinated 24 hours after anthesis. The fruits were all wasps, although sufficient tissue had been produced in the constricted portion so that they could be classed as first grade but not fancy. The types shown in Figure 2, A, are characteristic. Seeds developed in one-half to two-thirds of the embryo sacs.

Bed No. 2. No nitrate of soda added.

- A. Flowers pollinated at anthesis. Sixty-three out of the first 100 fruits were practically normal while the others showed sufficient irregularities to be classed as seconds or culls. Seven of the 100 were bad wasp types, and very much under size.
- B. Flowers pollinated 24 hours after anthesis. All of the fruits were wasp shaped, varying from very short to normal length. All were seconds or culls. The fruits shown in Figure 2, B, C, and D, were common types.

Late pollination apparently accomplishes fertilization in the ovules of the tip of the ovary but not in the ovules toward the base. Sufficient tissue is produced to inclose the seed cavity, and only when the plant receives ample nutrients to fill out the fruit does it resemble the ideal for the variety.

As a result of the above observations further studies on pollination were conducted. A few preliminary notes are of interest.

The flowers on a cucumber plant are fully open between 2 and 4 o'clock in the morning at a temperature of 16° C. At 12° the flowers open very slowly and reach their maximum between 8 and 9 o'clock in the morning. Thus the time of anthesis can be controlled by the temperature.

Germination studies showed that pollen loses its vitality by 2 o'clock in the afternoon at temperatures of 27° C. in bright sunlight. Lower temperatures extend the time somewhat.

An attempt was made to bring about fertilization by pollinating at various times before and after anthesis. The night temperature was kept at or above 14° C. during the experiment. All pollinations, designated "at anthesis," were made between 6 and 8 o'clock in the morning, which was a few hours after anthesis for some flowers. The soil was sufficiently fertile to produce well-shaped fruits with ordinary pollination. The fruits and fertilized ovaries were all removed previous to starting the experiment and all unopened flowers were bagged. Although the results as shown are more or less fragmentary, they are indicative of the effect of time of pollination on the shape of the fruit.

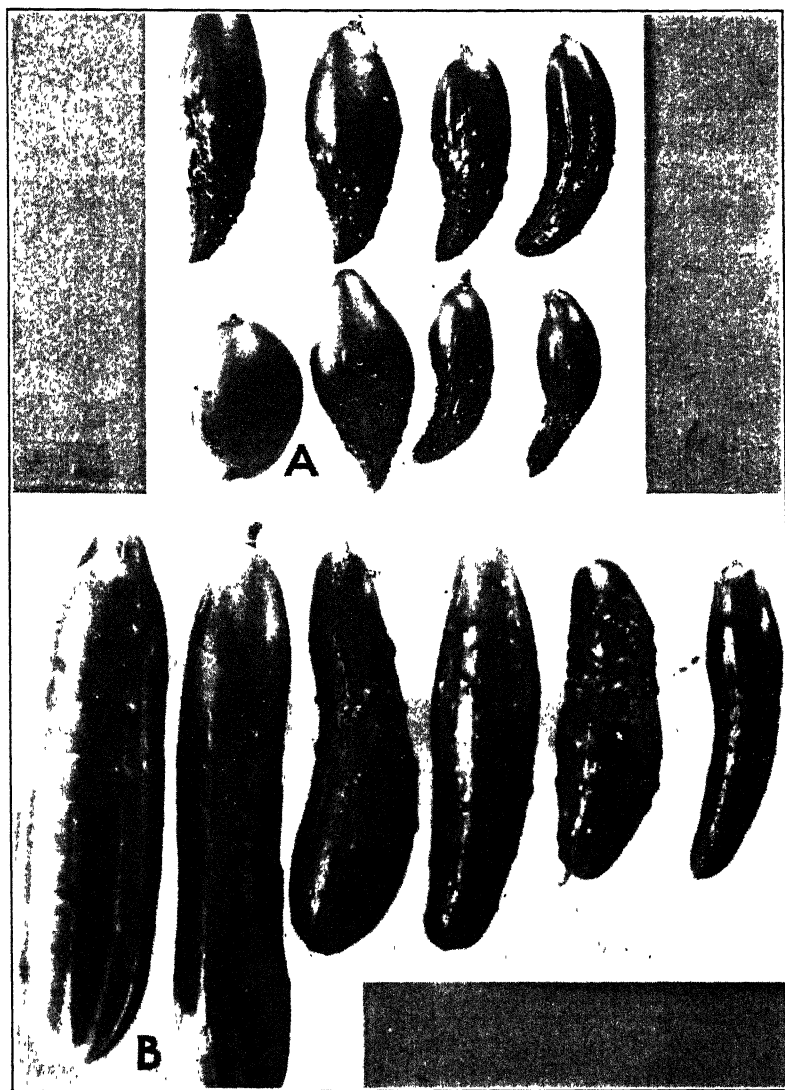


FIG. 3.—A, Nubbins produced by cucumber vines in poor vigor; B, stunted fruits, showing the detrimental effect caused by a growing fruit on the development of later-pollinated flowers

Pollen taken from staminate flowers—	Pollen deposited on pistillate flowers—	Percentage of pollinations successful	Shape of fruit
5 hours after anthesis.....	28 hours after anthesis.....	77	All wasp shapes.
Do.....	5 hours after anthesis.....	100	All first grade or fancy.
Do.....	18 hours before anthesis.....	70	All first grade.
Do.....	42 hours before anthesis.....	7	Do.
20 hours before anthesis.....	5 hours after anthesis.....	83	Do.
12 hours before anthesis.....	24 hours after anthesis.....	67	All wasp shapes.
20 hours before anthesis.....	20 hours before anthesis.....	0	
14 hours after anthesis.....	14 hours after anthesis.....	0	
8 hours after anthesis.....	8 hours after anthesis.....	92	17 wasp shapes out of 50 fruits.

The results show the possibility of using flowers before anthesis where a limited number of plants are being employed for genetic studies. Even though pollination is made some time before anthesis, the germination of the pollen probably does not take place until shortly before the beginning of anthesis when the sugary secretion is produced. The age of the pollen then determines whether fertilization will take place and may account for the low percentage of set where the pistillate flower was pollinated 42 hours before anthesis. The pollen grains are mature at least a day before anthesis so that they may be transferred to the stigmas, but the anthers must be broken to liberate them.

#### RELATION OF AMOUNT OF POLLEN TO SHAPE OF FRUIT

To determine the amount of pollen necessary to bring about fruit development, pollen grains were collected on the point of a dis-

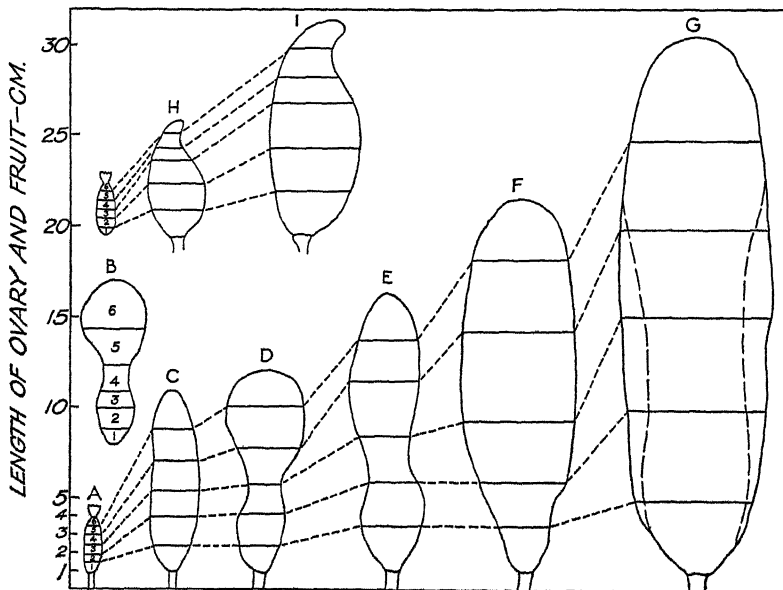


FIG. 4.—Comparison of growth of various parts of the ovary in fruits of different shapes. Seeds in fruits B and D to G are in the same stage of maturity. The difference in shape of the fruits is due to the time and duration of interruption in the development of the ovary

secting needle and counted under a dissecting binocular. A known number were placed on the stigmas of flowers which had been covered previous to anthesis. The pollinations were made after all developing fruits and fertilized ovaries were removed. The fruits were permitted to grow and the seeds were counted when mature. The plants varied considerably in vigor.

The results showed that in no case did the number of seeds exceed the number of pollen grains placed on the stigmas. The placing of pollen grains on one or all three of the lobes of the stigma did not change the location of the seed in the mature fruit. There was no correlation between the number of seeds produced and the shape of the fruit. A fruit with 2 seeds was as well shaped as one with 200 seeds, provided the fruit was grown on a vigorous vine. A large

number of nubbins shown in Figure 3, A, were produced on vines in poor vigor.

#### EFFECT OF DEVELOPING FRUITS ON THE SHAPE OF LATER-POLLINATED FRUITS

The detrimental effect of a developing fruit on later-pollinated flowers was indicated by such types as are shown in Figure 3, B, which when mature were stunted, with a premature yellowing of the shoulders. In order to determine the effect of dwarfing of the fruit on the subsequent growth of its various parts, small rubber bands were placed at equal distances on a number of small fruits, some of which were growing normally while others had had their growth cycles interrupted at various stages. Daily observations were made. Figure 4 shows the amount of growth produced in various parts of the small fruit in different types of fruit. The fruit are drawn to scale for comparison.

In Figure 4, A represents an ovary at anthesis, while B is a stunted fruit the ovary of which did not begin to grow until 22 days after pollination, when only the two sections in the tip developed seeds. C grew immediately after pollination and represents an ovary eight days after pollination, which is a critical stage for the embryos in the ovary. A period of inhibition<sup>5</sup> will mature many of the young embryos if prolonged for any length of time. The location of the matured embryos depends on the stage at which inhibition exerts its influence. An ovary eight days after pollination will begin to exert an inhibitory effect on later-pollinated flowers if the food in the plant becomes scarce. Figure 4, D, E, and F, shows the effect of stopping the growth of the ovary at the stage illustrated in Figure 4, C, for 15, 9, and 5 days, respectively. Maturity of the tissue begins at the base and increases toward the tip as the period of inhibition is prolonged. Figure 4, G, represents a normal fruit with a wasp type outlined to show this effect without a decrease in size. Fruits in Figure 4, B and D to G, have seeds at the same stage of maturity; H and I show the region of growth of two nubbins. In the nubbins the growth rate is retarded so that the ovules in the base of the small fruit develop at the expense of the ovules in the tip portion and there is a corresponding growth of the regions in the base of the small fruit instead of in the tip portion as is true in the wasp type of fruit. It is reasonable to suppose that ovules in all parts of the ovary become fertilized.

#### GROWTH PERIOD OF OVARIES AND FRUITS

Daily measurements were taken on a large number of developing fruits for length and diameter, to determine the variation of growth curves caused by external influences. These measurements were begun at anthesis and continued until maturity of the fruit. In Figure 5 the growth curves for fruits produced on typical, heavy-set plants are shown, together with the corresponding outlines of these fruits. In Figure 6, A, the main stem of this plant is shown with numbers indicating the location of the fruits corresponding to the numbers in Figure 5.

<sup>5</sup> In these studies inhibition is considered to be the effect whereby a maturing fruit prevents the growth of later-pollinated flowers.

At a glance it will be seen that the growth of the ovaries is interrupted at various periods after fertilization and that the production of fruit on the vine is divided into very definite periods. During each period two or three fruits matured, the removal of which gave an impetus of growth to a large number of ovaries and small fruits. The number of ovaries that develop normally depends on the amount of available food in the plant. A comparison of the shapes of fruits

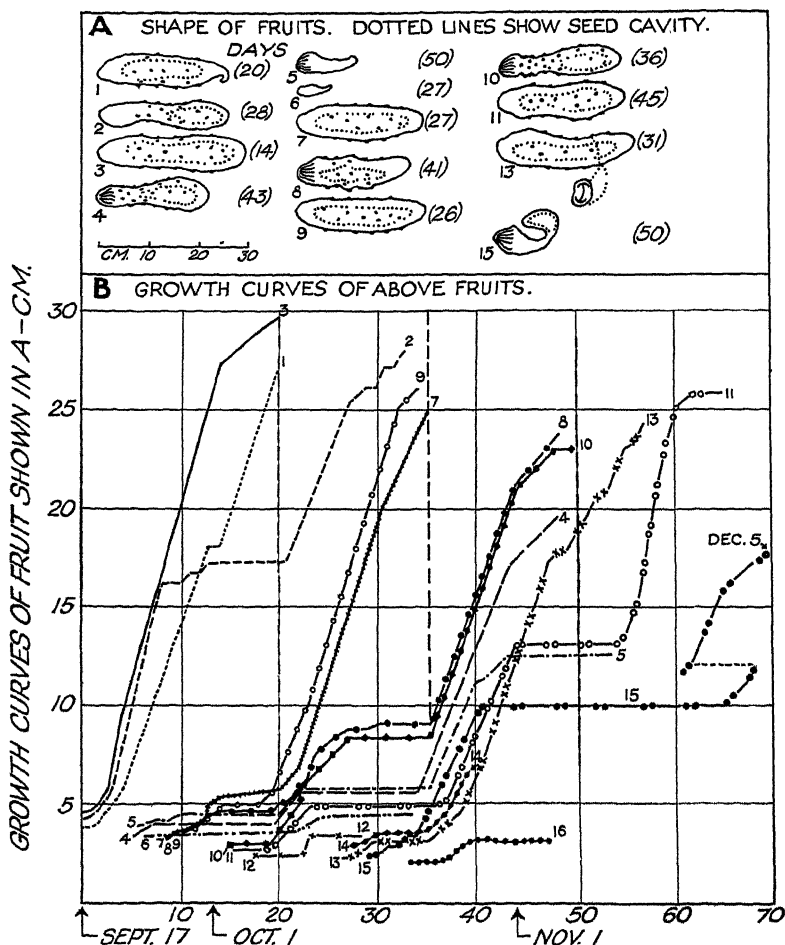


FIG. 5.—A, shape of fruits and number of days from pollination to maturity; B, growth curves for each of 16 fruits produced on one cucumber vine from September 17 to December 5

2, 4, 10, and 11 in Figure 5, with their respective growth curves, show that the wasp type may also result from an interruption in the growth cycle of the fruit.

An examination of the seeds showed that the ovules had partially developed in the constricted region of those fruits in which the interruption of the growth cycle of the fruit occurred five or more days after fertilization had been accomplished. Viable seed was found only in

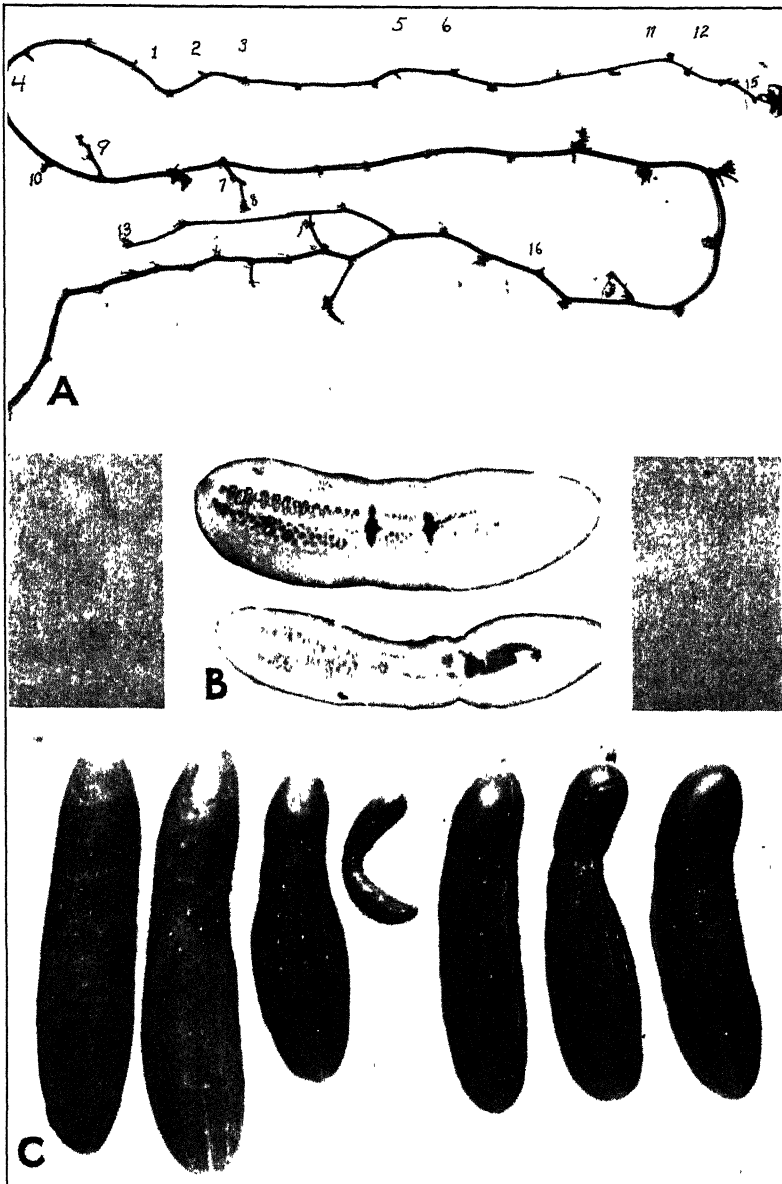


FIG. 8.—A, Main stem of cucumber plant the fruits of which are shown in Figure 5. The fruits and the nodes at which they were borne bear corresponding numbers. B, Fruits showing pithy and hollow centers caused by interruption in growth. C, Fruits in which the wasp shape is caused by inhibition of growth.

the bulbous portion of the fruit. In fruit 10, Figure 5, which had been interrupted twice before reaching a length of 8 cm., a hollow pithy condition resulted, similar to that shown in Figure 6, B. No development of the embryos was visible in the constricted portion. The ovaries of fruits 4, 5, and 6 stopped growing before fertilization

could be accomplished. Fruit 4 developed the characteristic wasp shape, while 5 and 6 dried up before maturing any seeds. In Figure 6, C, a number of fruits from another vine are shown in which the wasping effect is due to the inhibition of growth. The effect on the size of the fruit is evident.

The location of the fruits on the vines has some relation to their final size and shape, as is shown in Figure 5. Fruits 7 and 9 located on laterals had an advantage in the second period over 4, 5, and 6 on the main stem. The prolonged interruption accounts for the inability of fruits 5 and 6 to recover from the period of inhibition. Number 7 had an advantage over 8 because the general flow of food from the main stem into the lateral reached 7 before it reached 8. Fruit 13, pollinated 10 days after 11 on the main stem, had an advantage because of its location on a lateral and apparently inhibited 11 from growing in the fourth fruiting period, even though it was a distance of 41 nodes away. General flow of plant food seems to be the only explanation for inhibition of growth and even this seems unsatisfactory.

Plants having more laterals and fewer pistillate flowers on the main stem do not show such contrasting results because fewer fruits develop at one time and there is a greater amount of leaf surface for each ovary.

#### EFFECT OF LIGHT ON THE DEVELOPMENT OF FRUIT

In Figure 7, A, are shown a number of fruits, on plants grown from December to February, subjected to different conditions of light. The plants from which the four large fruits in the top row were taken received electric light in addition to sunlight from two hours after midnight to sunrise; the four large fruits in the bottom row were taken from the control plants; and the six small fruits on the right were taken from plants shaded from 3 o'clock in the afternoon until 9 o'clock the following morning. Although the electric light (100 foot-candles) increased the size of the fruit and the number of seeds slightly, the greatest difference occurred in the shaded plants where fertilization of the ovules did not take place even though viable pollen was used. No seeds were produced in these fruits. The plants also were somewhat weaker than the controls. Although the shaded plants received sunlight during its greatest intensity for 6 hours out of a possible 10 hours of daylight, seed formation was entirely inhibited. Reducing the sunlight stimulated the production of seedless fruits on vines which would not produce fruit without fertilization during days of maximum sunlight. The parthenocarpic condition found in English varieties was induced in a nonparthenocarpic strain by changing the nutrition of the plant.

#### DISCUSSION

The data show that anything interfering with the development of the embryo affects the shape of the fruit, the degree of deviation from the normal depending on the number of ovules which fail to produce viable seed and the amount of tissue produced around the seed cavity. The growth of the seed in any portion of the ovary causes that portion to bulge or fill out. Uneven distribution of seed necessarily produces an irregularly shaped fruit.



## LATE POLLINATION

Flowers pollinated one or two days after anthesis produce wasp-shaped fruits because only the ovules near the stigma produce seed. This may be due to some change in the nutrition of the ovary so that



FIG. 7.—A, Cucumber fruits on plants grown from December to February under different conditions of light. The four large fruits in top row taken from plants receiving electric light in addition to sunlight from two hours after midnight to sunrise; the four large fruits in bottom row taken from control plants; the six small fruits on the right taken from plants shaded from 3 p. m. to 9 a. m. B, a, Cucumber seeds from a fruit developed parthenocarpically during days of minimum sunlight; b, seeds from a fruit interrupted in its growth; c, seeds from a normal fruit

the pollen tube finds it difficult to reach the ovules in the base, or to the fact that the ovules have passed the receptive stage. The latter probably depends on the nutritional condition in the ovary.

The degree of constriction depends on the amount of tissue that the plant forms around the seed cavity. Apparently the growth

of the various regions of the ovary is not interfered with, although seeds are produced only in a portion of the fruit. If late pollination is associated with inhibition of the growth period, then of course the length of the fruit will be reduced. If there is a surplus of food available for the growth of the fruit, the visible wasping effect may be completely obliterated even though the seeds develop in only a portion of the fruit.

#### INTERRUPTION OF THE GROWTH PERIOD

Flowers pollinated simultaneously at the same node develop as twins and are very uniform in size and shape, although they may not resemble the type characteristic for the plant. If a flower is pollinated one day earlier than another, it exerts an inhibitory effect on the flower subsequently pollinated when the supply of food in the plant is not sufficient to mature all the fertilized ovaries. This inhibitory effect is exerted as long as the fruit remains on the vine. If this period is not too long, the later-fertilized ovaries resume their growth. The whole phenomenon is correlated with the amount of food that is available in the plant at one time.

In flowers at a node pollinated simultaneously, the food is divided evenly among them, as is shown by the uniformity in size, shape, and the time of maturity of the fruit. In flowers pollinated at different times there is an uneven distribution of food, and the first-pollinated flower receives the advantage. The shape of the fruits resulting from this inhibitory influence shows a direct correlation between the length of the inhibitory period and the degree of constriction of the fruit. Fruit No. 15 shown in Figure 5 is a good example of the maximum time that the growth of the ovary may be inhibited without dying and yet produce some viable seeds. This flower was pollinated on October 28, grew very slowly for 7 days, grew normally for 7 days, was inhibited for 24 days, resumed growth, and matured five seeds on December 5. For 50 days the deformed fruit remained on the vine and matured seeds after being practically dead at the end of the period of inhibition. It was the only fruit on the vine after the tip resumed growth.

The effect of this inhibition on the shape of the fruit is evident and has considerable application to practices followed by the grower in producing fancy fruit. Whether the fruit is a nubbin (fig. 3, A) or whether it is a wasp (fig. 2, C) depends on the stage of development of the embryo when inhibition takes place. These fruits are practically opposite in type of development, due to the time at which inhibition exerted its influence.

When three flowers are pollinated simultaneously at different nodes there is usually sufficient food available for their development and normal growth. If the food becomes depleted to the point where one of the small fruits slows down in its growth the ovules in the base have developed sufficiently so that they continue to grow at the expense of those in the tip. Starvation gradually causes a dwarfing of the fruit. The food may be curtailed for the second fruit, but it will become much larger before dwarfing takes place. Curtailing the food supply results in nubbins of various sizes. Food apparently is directed into one ovary in preference to another. The location on the plant may determine their final size. In the opposite

type where wasping occurs there is an abrupt stop in the growth period. Can one say that the food is abruptly stopped in its flow to these fruits in favor of others? To do so might almost give the plant credit for directive growth of the ovaries. Were the slowing-up process gradual it might be attributed to starvation.

If food is suddenly made available by the removal of a developing fruit, a nubbin may resume growth and reach considerable size by an extension of the regions in the tip. In the wasp fruit, growth is resumed in the region where the seed is developed so that the fruit does not materially increase its length if the period of inhibition has been much prolonged.

An examination of a large number of these different abnormalities shows that in flowers pollinated at anthesis, if the embryos reach a certain size by uninterrupted growth immediately following fertilization, a period of inhibition of considerable duration will not prevent the larger ones from resuming growth. The larger embryos will be near the base of the ovary and account for the formation of nubbins. The two fruits on the left in the lower row in Figure 3, A, are characteristic of the type of fruit in which the period of inhibition was sufficiently prolonged so that the embryos in the tip did not resume growth.

If the period of inhibition follows immediately after pollination, fertilization of the ovules in the base of the ovary may not be accomplished. The flow of plant food into the ovary is stopped so that the pollen tube is unable to grow because of insufficient nutrients. Only the ovules in the tip close to the stigma become fertilized if any fertilization takes place. A prolonged period of inhibition may not prevent the embryos in the tip from developing when inhibition is removed.

The ovary increases in size very slowly the first few days after fertilization. If growth is not inhibited until after fertilization of the ovules, many probably become matured in the base of the ovary, because they do not resume growth after a period of inhibition. The seeds in Figure 7, B, c, were taken from a normal fruit, those in Figure 7, b, from a fruit which was interrupted in its growth, and those in Figure 7, a, from a fruit developed parthenocarpically during days of minimum sunlight. The sterile seeds in b show the effect of inhibition of the growth of the ovary after fertilization has taken place.

#### LIGHT CONDITIONS AND SHAPE OF FRUIT

During days of maximum sunlight a flower must be properly fertilized before growth of the ovary takes place. There is also less tendency for fruits to develop parthenocarpically. A fruit which has been inhibited in its growth rate will mature much quicker and produce very characteristic "yellow pickles."<sup>6</sup> As the amount of sunlight decreases in intensity and duration, there is a gradual change toward what might be termed a "vegetative" response. Less stimulation is necessary to start fruit development and many plants will produce parthenocarpic fruits. The yellow-pickle condition becomes less pronounced because of the prolonged life of the tissue in an inhibited fruit. Yellowing of the base of the fruit is much slower. The

<sup>6</sup> TIERJENS, V. A. YELLOW PICKLE IN GREENHOUSE CUCUMBERS. Mass. Agr. Exptl. Sta. Bul. 225, 8 p. 1925.

condition leads to more abnormally shaped fruits during days of reduced sunlight because ovaries which produce yellow pickles during days of maximum sunlight become sufficiently developed during days of minimum sunlight to be classed as seconds. Some of these are shown in Figure 3, B. There is a slight yellowing of the shoulders of small fruits due to maturity of the tissue before the seeds mature in the tip of the fruits.

The whole problem resolves itself into one of carbohydrate starvation. Reducing the light reduces the carbon compounds so that nitrogen metabolism may be modified. Apparently the surplus of sugar has much to do with the inhibition of parthenocarpic fruits during days of maximum sunlight. To say that tissues are matured more quickly by sufficient carbon for skeletal structures would be merely a hypothesis, but such seems to be the case.

#### RELATION OF SHAPE AND SIZE OF FRUIT TO GENETIC STUDIES

It has been shown that conditions of environment have a profound effect on the shape and size of mature fruits. Many studies in size inheritance involve the measurement of fruits for data indicative of the genetic potentiality of a plant. In Figure 6, C, are shown a few fruits of a single cucumber plant taken at different times in its life cycle. Inhibition in the growth of the fruits has had a tremendous effect on their shape and size.

The practice of taking data on size of the mature fruits of a plant to obtain an average or mean for the genetic potentialities seems inadequate. A big-fruited plant may be classed as intermediate because of environmental factors which reduce the size of its fruit. More reliable data would result if observations were taken from the maximum-sized fruit produced by the plant because these data would really indicate the potentialities of the plant for size of fruit. This could be accomplished by limiting the controlling factors to a minimum. Thinning fruit on the vine to the number that the plant will mature successfully would give much more reliable information than the present practice, even though the results were based on relatively few fruits.

Averaging of fruits for size makes it difficult to correlate size with other factors because size is variable. If a maximum-sized fruit for the plant is used, it becomes much less difficult to study linkage between size and other characters. As an example, the writer was interested in correlating size of fruit with certain vegetative characters. By using the maximum size of fruit for the plant very good linkage could be shown, while the average of all the fruits showed no linkage because the average for a plant was too close to the average for the entire population. This was especially true of the long-fruited selections. The longest fruit on one plant was 42 cm., while the average or mean length of its 27 fruits was 34.5 cm. This discrepancy in length was due to the various conditions discussed in this paper. The distribution curves for the short-fruited types are quite narrow, so that the mean is very close to the maximum size for the plant. Thus, maximum length seems to be the logical figure to use as typical for the cucumber fruit to expedite genetic research in size inheritance and its linkage relations to morphological characters.

## CONCLUSIONS

Nubbins are fertilized ovaries gradually starved after their embryos have developed to a certain stage without absolute inhibition of growth. The cause of the "wasp," in addition to delayed pollination, is due to absolute inhibition of growth by prevention of fertilization or by maturation of the fertilized ovules before the embryo has reached sufficient size to recover. It is, then, a question of determining the relation of the inhibition phenomenon to starvation, which is not clear at the present time. Starvation seems to be a gradual process, while inhibition is abrupt. Both processes may be fundamentally the same.

From the results obtained by increasing and decreasing the light increment, the availability of carbohydrates in the plants seems to have a tremendous effect on the shape of the fruit and fertilization of the ovules. The surplus of sugars in the plant probably has some bearing on whether the fruit is gradually stopped in its growth period or whether it is abruptly inhibited. The data from the shaded plants suggest some relation between duration of periods of illumination and darkness. The six hours of illumination probably were so much out of proportion to the hours of darkness that the nutrient balance was completely upset.

In a study of size inheritance and its linkage relations to morphological characters in the cucumber, it is recommended that instead of averaging fruit for size, as is commonly done, the observations be taken from the maximum-sized fruit produced by the plant.



# FROG-EYE LEAF SPOT OF SOY BEAN CAUSED BY *CERCOSPORA DIAZU* MIURA<sup>1</sup>

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## INTRODUCTION

The soy bean, *Soja max* (L.) Piper, is subject to a number of diseases which are manifest primarily in the form of leaf spots. Some of these which have somewhat recently been described are bacterial blight (3, 11),<sup>2</sup> bacterial pustule (5, 12), mildew (7), and brown spot (13). Lately another leaf-spot disease new to America has appeared on soy bean and is already widespread in the South. As a matter of convenience for reference, as well as appropriateness of symptoms, the name "frog-eye" will be used as a common designation of this disease. It is the purpose of the present paper to give a brief account of frog-eye leaf spot of soy bean and to describe the organism which causes it.

## HISTORICAL ACCOUNT

Except for a few earlier and very brief statements (14)<sup>3</sup> regarding the occurrence of the disease herein described, there has appeared as yet in America no account of the active parasitic attack on soy bean by any fungus in the genus *Cercospora*. In 1901 Carver (2, p. 5), in publishing a list of 75 species of *Cercosporae* found in Macon County, Ala., noted the occurrence of a fungus, which he designated *Cercospora canescens* E. & M., on soy bean and several other unrelated hosts. This appears to be the earliest reported occurrence of a *Cercospora* on soy bean in America. The occurrence only was noted, however, and no description of the symptoms of the disease was given. The writer has not seen specimens of this collection, but it appears from Carver's description, as given in correspondence and from examination of similar specimens collected and sent to the writer during the summer of 1927, that *C. canescens* is saprophytic or at most only weakly parasitic on soy-bean leaves. Certainly the appearance of soy-bean leaves bearing *C. canescens* is symptomatically very unlike that of leaves attacked by the frog-eye disease. Likewise, the morphology of the organisms associated with the two diseases is very different.

In 1921 Miura (9) in a brief mycological note published a description of a *Cercospora* leaf-spot disease of soy bean which he had found at Tu-men-ling, south Manchuria, August 19, 1918. He ascribed it to a new fungus, which he named *Cercospora diazu*. The same disease was observed in Japan in 1924 by Hemmi, and diseased material was sent to F. A. Wolf, along with other specimens

<sup>1</sup> Received for publication Mar. 5, 1928; issued July, 1928. Published with the approval of the Director of the North Carolina Experiment Station as paper No. 25 of the journal series.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 832.

<sup>3</sup> HASKELL, R. J. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1925. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr., Sup. 48: 376. 1926. [Mimeographed.]

of soy-bean diseases of the Orient. The North Carolina leaf spot was reported by Wolf and Lehman in 1926 (14) as identical with that found by Miura in Manchuria in 1918.

In 1924 Moore<sup>4</sup> recorded the occurrence of a *Cercospora* on soy bean in South Carolina. The disease was present on about 20 per cent of the plants in one field at Clemson College. No specimens of this collection are available for study, and the identity of the disease remains in question. It seems probable, however, as indicated below, that this may well have been the first reported occurrence of the frog-eye leaf spot of soy bean in America.

In 1925 Weimer<sup>5</sup> collected specimens of a soy-bean leaf spot at Clemson College and Orangeburg, S. C., Starkville, Miss., and Baton Rouge, La. In 1926 Moore again collected specimens of a *Cercospora* leaf spot on soy bean from a section of South Carolina remote from Clemson. Representative specimens of the 1925 collection by Weimer in South Carolina and the 1926 collection by Moore have been examined by the writer and identified as frog-eye.

Under date of August 15, 1925,<sup>5</sup> C. W. Edgerton reported the occurrence of a *Cercospora* leaf spot of soy bean in Louisiana and gave 1925 as the first year in which the disease had been observed in that State. Through the kindness of Edgerton the writer has been able to examine specimens of the Louisiana disease and regards it as identical with that found by Miura in Manchuria in 1918 and further described by the writer in the present paper.

Frog-eye of soy bean was first seen by the writer on September 23, 1925. At that time the disease was observed in a variety planting near Moyock, N. C. Lesions were very numerous on leaves of the Laredo and Ootootan varieties. As far as the writer is aware, this observation constitutes the first knowledge of the presence of the frog-eye disease in North Carolina.

The brief historical account given above includes all reports of the occurrence of frog-eye on soy bean which have come to the writer's attention. It is evident, however, that more than one species of the genus *Cercospora* occurs on soy bean. This conclusion is supported by the writer's experience in September, 1923, when a hitherto unreported occurrence of a *Cercospora* on soy bean was observed in the experimental plots at Raleigh, N. C. The aspect of the disease was entirely different from that of frog-eye. The fungus had spread over rather large areas of the leaf and was not limited to definitely bordered spots. The leaf tissues were more or less moribund, a condition which may have preceded or may have been caused by the attack of the fungus. Although the fungus was examined microscopically, its species was not determined nor have specimens been preserved. Again in the summer of 1927 the writer observed a *Cercospora* fruiting on dead areas in living soy-bean leaves. In this case it appeared that the tissues had first been killed by confluence of lesions of the bacterial-pustule disease and that the *Cercospora* had come in as a secondary invader. Microscopic examination of this fungus showed it to be morphologically much like *C. canescens*.

<sup>4</sup> MELCHERS, L. E. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr., Sup. 40: 186. 1925. [Mimeographed.]

<sup>5</sup> HASKELL, R. J. Op. cit.



In the summer of 1927 a lavender spot disease of soy-bean seeds was found in several fields in North Carolina. Although the writer has not been able as yet to determine definitely the identity of the causal organism, the trouble is symptomatically much like the disease described in Japan as due to *Cercospora kikuchii* Mat. & Tomo. (8). Experimental evidence thus far obtained indicates that the lavender seed-spot fungus in North Carolina is specifically different from that which causes frog-eye.

#### DESCRIPTION OF FROG-EYE LEAF SPOT

Frog-eye leaf spot is primarily a foliage disease. Lesions have never been observed as yet on pods, and in only one instance have they been found on stems. On leaves, diseased spots arising from single infection centers vary in size from mere specks up to 5 mm. in diameter. The spots are usually discrete, but occasionally several coalesce, forming diseased areas as large as 10 mm. in diameter. The sizes most commonly observed fall within the limits of 1 to 3 mm.

The symptoms in the early stages of infection vary somewhat with different varieties of soy bean and with leaves grown in a moist or dry environment. On leaves of the Laredo and Oototan varieties the lesions first appear on the up-

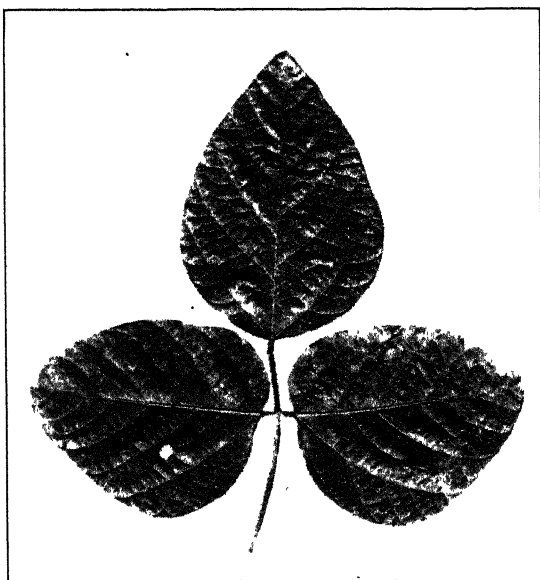


FIG. 1.—Very young lesions of frog-eye on a leaf of the Laredo variety of soy bean brought from the field September 27, 1927. The lesions are rather numerous on each leaflet, and the largest of them have begun to show the light centers. Actual length of the tip of leaflet  $2\frac{1}{2}$  inches

per side as minute reddish brown spots varying from 0.25 to 0.5 mm. in diameter. (Fig. 1.) At this stage the color on the lower side of the diseased leaf differs but little from that of normal leaf tissue, being only slightly reddish or merely a different shade of green.

By the time the diameter has increased to 0.8 mm. the reddish-brown color usually begins to fade from the center of the spot but remains at the margin of the lesions as a narrow reddish-brown border. (Fig. 2.) This color in turn passes abruptly into the green of the normal leaf without the intervention of a zone of chlorotic tissue. As the spot continues to enlarge, the reddish-brown band or border moves outward, always remaining very narrow and seldom exceeding 0.25 mm. in width. (Figs. 2, 3, and 4.) The central light-brown area enlarges apace, becoming still lighter, so that in the larger, fully developed spots the central diseased tissue is Hay's russet,

cinnamon buff, olive gray (10), or ash gray in color. At this stage the color of the diseased tissue is usually somewhat darker brown or darker gray on the lower side than on the upper side of the leaf and a given spot may show clearly a marginal dark-brown border on the upper side while this is not at all or only very faintly in evidence on the lower side. The disappearance of the green color and the

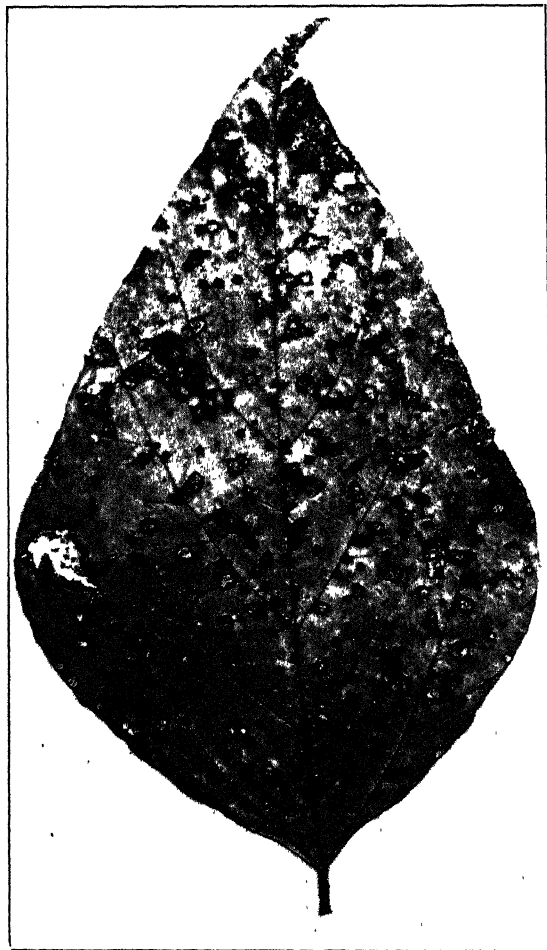


FIG. 2.—Fully developed lesions on a leaflet of the Laredo variety of soy bean brought from the field September 28, 1925. These lesions show light brown, ash gray, and almost white centers surrounded by a narrow reddish brown marginal border as they appear on the upper surface. This leaf is from the first collection of frog-eye in North Carolina

development of the brown and gray shades go on more slowly on the lower side of the leaf than on the upper side. This difference in color is often enhanced by the presence of the fructifications of the causal fungus. Dark-colored conidiophore clusters develop on both sides of the leaf, but are usually fully twice as abundant on the lower as on the upper side. These fascicles of conidiophores are usually sufficiently prominent to constitute a definite sign by which the disease may be recognized unmistakably with the naked eye, or, at most, only a hand lens is needed to distinguish them from fructifications of other fungi parasitic on soy bean. They develop in the center of the lesion (fig. 5), and their dark color when massed obscures the light-brown or ash-gray color characteristic of the diseased tissues in the center of the spots,

and thus the lower side of a lesion may appear upon cursory examination to be much darker than the upper side.

In older spots on which conidiophores have ceased spore production, the diseased tissue within the marginal brown ring becomes very thin, often paper white, and almost transparent. The dark-brown marginal ring is often raised above the general leaf surface, so that its thickness exceeds that of the adjacent normal leaf tissues.

In shape the spots arising from single loci of infection are roughly circular but irregular or angled spots are numerous (figs. 2, 3, and 4), the irregularity arising from growth difficulties when the causal organism encounters a veinlet. The spot develops in a uniform circular area within the areolae of the leaf, but whenever a veinlet is encountered growth is more rapid parallel to the veinlet than across it. Diseased areas arising from single loci of infection seldom if ever cross the midrib or the primary lateral veins of the leaf. Secondary lateral veins are frequently crossed, but even laterals of the third and fourth orders are traversed with some difficulty and usually give rise to more or less pronounced angularity of shape. Irregularity of shape is the rule when diseased areas arising from two or more loci of infection come in contact with each other. Because of the comparatively small size of the spots and the apparent inability of the causal fungus to destroy the vascular elements of the leaf, the diseased areas do not readily break up and fall out.

Frog-eye has been observed occurring naturally on stems in only one instance. This was in early October and on the variety Ootootan. Nearly 100 per cent of the leaves were infected, each leaf bearing from few to many lesions. Notwithstanding this high leaf infection, cauline lesions were few in number and could be found only on the young, less woody portions of prostrate shaded stems.

These diseased areas have averaged two to three times as long as broad, the lesions lying in the direction of the long axis of the stem. On young lesions, one-fourth to one-half inch long, the central and oldest portion of the diseased area is English or mahogany red (10) and is surrounded by a zone of black, beyond which is the normal green of the young stem collenchyma. As the lesion grows older, it enlarges and the central area loses its red color, becoming first brown then a pale smoke gray. A persisting band of red separates the gray center from the outer bordering band of black. Minute stromata, often bearing fascicles of conidiophores with conidia, are present in numbers on the gray centers.

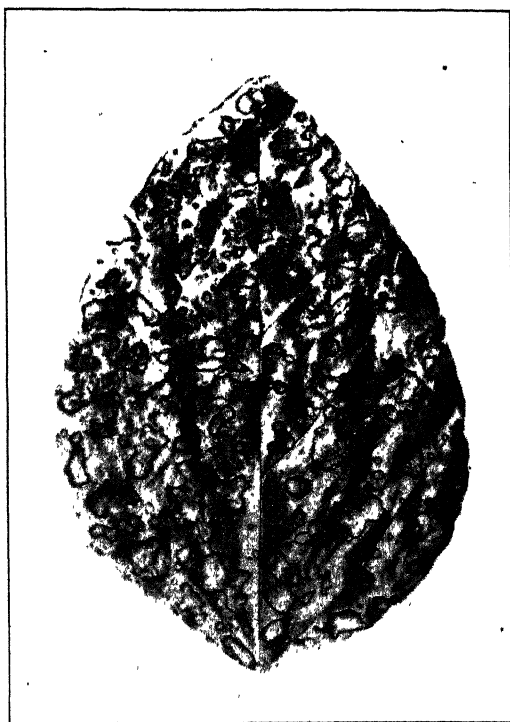


FIG 3.—Mature lesions on a leaflet brought from the field September 27, 1927. Note the high proportion of leaf area infected. Actual length of leaflet  $3\frac{1}{4}$  inches

Careful isolations from the stem lesions described above yielded unmixed cultures of a *Cercospora* morphologically and culturally like strains previously isolated from leaves. Moreover, lesions produced on leaves in artificial inoculation tests with strains of the fungus isolated from stems were in no characters different from those produced by strains obtained from leaves.

Frog-eye is usually sufficiently well marked by characters peculiar to itself to be distinguished from other leaf spots of soy bean on the basis of symptoms and signs macroscopically visible. The brown specks representing the first stage of the disease resemble lesions produced by bacterial blight (*Bacterium glycineum* Coerper, *Bact.*

*sojae* Wolf), but differ in that the latter are darker in color and decidedly more angular and are usually surrounded by a zone of chlorotic tissue, the last-mentioned symptom being entirely absent in frog-eye lesions. Frog-eye may be distinguished from bacterial pustule (*Bact. phaseoli* var. *sojense* Hedges) by the absence of the pustular outgrowths and chlorotic halos present in the early stages of the latter disease and by the absence of the uniform rusty-brown aspect characteristic of the later stages of bacterial pustule. In the case of brown spot (*Septoria glycines* Hemmi) of soy bean the spots enlarge up to 4 to 5 mm. but

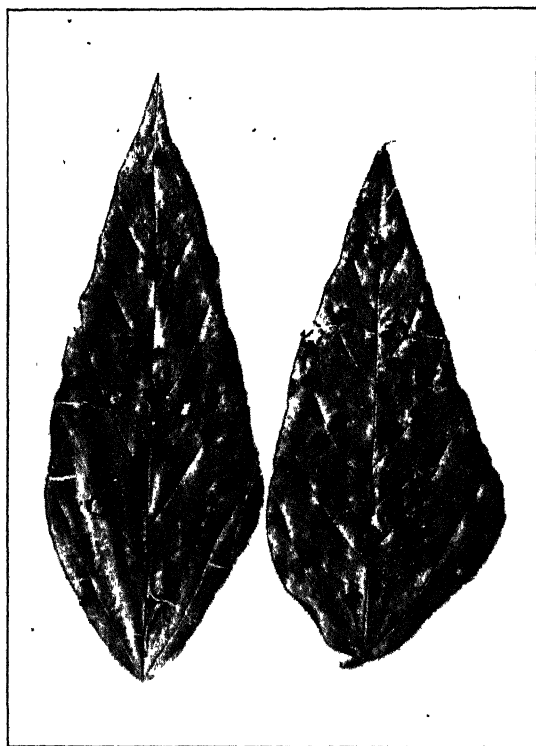


FIG. 4.—Mature lesions on leaflets of Ootootan collected by W. D. Moore in South Carolina in 1926. About three-fourths natural size

remain a uniform dark brown, reddish brown, or light brown according to the age and stage of pathogenesis and never develop spots with light-colored centers and reddish-brown borders characteristic of the frog-eye leaf spot. The mature spots of downy mildew (*Peronospora sojae* L. & W., *P. manshurica* (Naoumoff) Sydow in litt.) are symptomatically similar to advanced lesions of frog-eye, but the two diseases may be separated readily on the basis of the characters of their early developmental stages. A relatively large spot exhibiting mild chlorosis with no brown border represents the early stage of mildew; while in frog-eye lesions of this size the reddish-brown border is always present and the central tissues are dead, very light brown, ash gray, or even paper white. Mature

spots of mildew are nearly always larger than frog-eye. Moreover, the mature spots may readily be separated on the basis of fructifications present on them. In mildew they occur only on the bottom side of the leaf and have a grayish or violet woolly aspect, while in the frog-eye disease the conidiophores occur on both surfaces of the diseased areas and have a brown to black aspect. These signs can usually be so easily recognized by the naked eye that the two diseases may be separated correctly.

#### DISTRIBUTION OF THE DISEASE

Since the discovery of frog-eye at Moyock, Currituck County, N. C., the disease has been observed in a total of 12 counties. These 12 counties are widely distributed over the coastal plain and piedmont regions, the territory producing the greater part of the soy-bean crop of the State.

(Fig. 6.) Outside of North Carolina the disease has been found in four States—namely, South Carolina, Georgia, Louisiana, and Mississippi. Correspondence with pathologists in all other of the



FIG. 5.—Portion of lower surface of diseased leaf enlarged 1.7 times in order to show more clearly the darkened central areas from which the conidiophore clusters arise. Note that the larger diseased areas are formed by coalescence of lesions arising from several points of infection

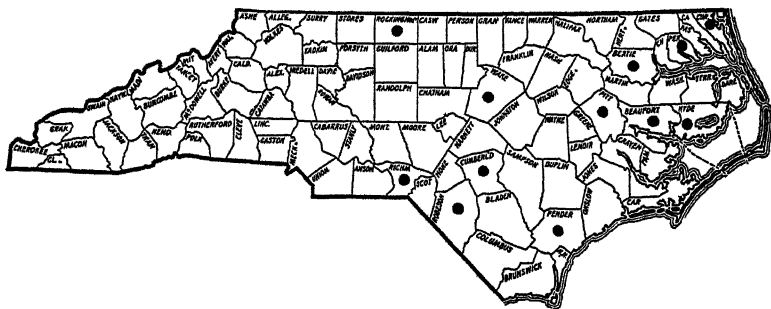


FIG. 6.—Map of North Carolina. The black dots indicate the counties in which frog-eye leaf spot of soy bean has been found

Southern States and in a number of Northern and Western States where soy beans are grown brought only negative reports regarding the occurrence of this disease. It is known to occur in Manchuria and Japan. These countries in all probability represent the region in which the disease originated and from which it spread to America.

#### ECONOMIC IMPORTANCE

In North Carolina the soy bean has taken rank as one of the main agricultural crops and is fast gaining in favor as a seed, forage, and soil-building crop throughout the South. In 1926 soy beans were grown on 159,000 acres in North Carolina and yielded seed valued at nearly \$4,000,000. Other States are recognizing the value of this crop and are rapidly increasing the acreage devoted to it. It is difficult to estimate correctly the reduction in yield or value due to such a malady as *Cercospora* leaf spot. The fungus kills the tissues attacked and thus reduces the photosynthetic activity of the leaf and possibly also adversely affects the water relations of the plant, a condition which may attain significance in dry seasons. Ordinarily the infected areas do not involve more than 5 per cent of the leaf tissue of a plant, and in such cases the losses may not be great. However, plants are frequently seen on which 25 per cent or more of the total leaf area is diseased. The spots may be so numerous as to give the plants a yellowish aspect. In such cases reduction in yield must be considerable. The disease is potentially capable of doing great damage. It certainly has not been with us long, and possibly, with the greater accumulation of infective material, losses resulting from it may be considerably greater than at present. This is particularly true since the varieties which appear to be most susceptible are now rapidly coming into favor.

#### VARIETIES ATTACKED

The disease has been observed on the following varieties: Laredo, Ootootan, Biloxi, Manchu, Mammoth Yellow, Goshen Prolific, Virginia, Austin, Tarheel Black, Wilson, Tokyo, Haberlandt, and Chiquita. Of this group, Ootootan and Biloxi are most susceptible; Chiquita, Tarheel Black, Wilson, and Mammoth Yellow are somewhat less susceptible. Early maturing varieties such as Dixie, Manchu, and Virginia escape serious injury; while such late maturing varieties as Ootootan and Biloxi suffer most.

#### ETIOLOGY

##### ISOLATION OF THE PATHOGENE

Diseased areas which have developed to maturity bear numerous conidiophores, each of which produces one to several conidia. Isolations are easily accomplished by the following method: Conidia scraped from the surface of a lesion are mounted in a drop of sterile water so as to make a dilute suspension. A loopful of this suspension is spread over the surface of a hard plain agar plate with a zigzag stroke. The spores germinate readily and in the course of five to six days thin gray colonies may be located and positively identified microscopically by observation of the conidia and conidiophores. If the isolations are made on plain agar from lesions on fresh, green

leaves, and the spore suspension is diluted, little difficulty is experienced in obtaining discrete colonies free from contaminating bacteria or fungi. Transfers may then be made from these colonies to other plates or to tubes.

#### DESCRIPTION OF THE CAUSAL FUNGUS

The fungus which causes frog-eye of soy bean sporulates abundantly by the formation of conidia and conidiophores on mature lesions. The latter occur in tufts or fascicles on both surfaces of the lesion but are present in greater numbers on the lower, less exposed side. The conidiophores composing a fascicle vary in number from 2 or 3 to as many as 25 and appear to arise from a stroma. (Fig. 7, A, B, C.) The stromata are always small and inconspicuous and often consist of little more than the aggregated basal cells of the conidiophores, but at other times there is evident development of stromal tissue. The stromata appear to arise on, in, or just beneath the epidermis and penetrate the leaf mesophyll to a depth equal to one-eighth to one-fourth the thickness of the leaf. Conidiophores viewed under apocromatic objectives are light brown (cinnamon) to dark brown (walnut brown) in color. They vary in length with favorable or unfavorable moisture conditions. Sizes ranging from 52 to 120 microns by 4 to 5.5 microns have been observed on specimens gathered from the field. They are usually 1 to 3 or 4 septate and show prominent geniculations and spore scars. (Fig. 8, H, I, J.) Each conidium is formed at the tip of the conidiophore (fig. 9, A, *a*, *b*), but the latter continues to elongate by pushing out at an angle from the original direction of growth. Thus it forms a kneelike flexure, grows forward (fig. 9, A, *b*, *c*; D, *d*, *e*, *f*) and produces another spore. When the spore falls away a scar is left at its place of attachment. (Fig. 9, D.) As many as 11 spore scars have been counted on a single conidiophore, the usual number being 1, 2, or 3. (Fig. 8, H, I, J.)

The conidia are multiseptate and hyaline, except that a few which have become light brown may be found at times on lesions which are several months old. They are elongate, fusiform, or tapering mostly toward the apex and less or not at all toward the base. The apex is rounded and about half as thick as the broadest part of the spore. The base is usually rounded and bears a circular scar marking the place of attachment to the sporophore. The septa vary in number from 0 to 10. The 3-septate forms are most numerous, 33 to 53 per cent of the spores falling into this group. The classes represented by 4, 5, 6, and 7 septate forms are approximately equal in point of numbers, each of these classes containing about one-fourth to one-half as many spores as the 3-septate group, or about 10 per cent of the total number of spores. On leaves the conidia range in size from 24 to 108 microns by 3 to 9 microns, the most common sizes being 40 to 60 microns by 6 to 8 microns.

Table 1 shows the distribution of the conidia according to the septation classes in which they happen to fall, and Table 2 shows the average spore sizes in the different septation classes. When grown in culture on plain agar, the 3-septate forms remain by far the most numerous. In culture, however, the average sizes of all the septation classes are larger than those occurring in nature. Spore measurements taken from plain agar cultures of a *Cercospora* isolated from

cowpea show an entirely different distribution in septation classes, and the spore sizes are smaller than for the soy-bean *Cercospora*.

Conidia collected at different times and places and on different varieties of soy bean are shown in the camera lucida drawings of Figure 8, A-G. Spores from the variety Biloxi drawn seven days after the leaves were collected are shown in Figure 8, A. Most of the spores were still turgid, but in many of them the protoplasts

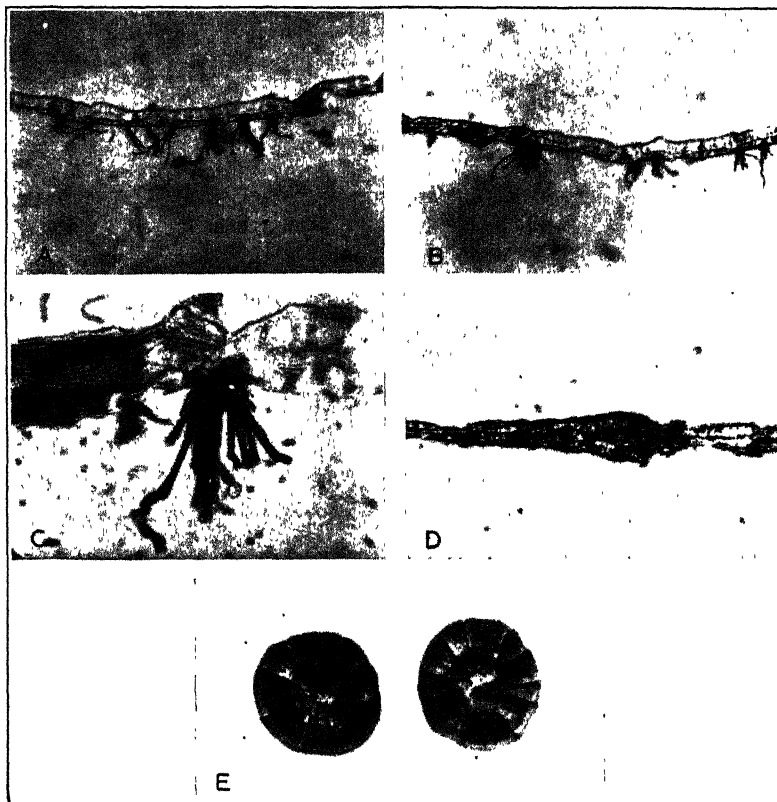


FIG. 7.—*Cercospora diazu* as it appears on lesions on soy bean leaves and in culture

A.—Paraffin section through the sporiferous area of a lesion. Note the clusters of conidiophores arising from the lower surface.

B.—Similar to A.

C.—A single conidiophore cluster enlarged to show septation and spore scars. Most of the conidiophores have broken off, but one with several spore scars is still intact.

D.—Paraffin section showing hypertrophied tissue in the region of the brown margin of the lesions. Undiseased tissue of normal thickness at right of the deeply staining, hypertrophied region; to the left is the direction of the center of the lesion.

E.—Colonies of *Cercospora diazu* growing on potato-agar plates incubated 15 days at 25° C.

in one to four cells had disintegrated, allowing partial collapse of the walls. The spores of Figure 8, B and C, were taken from different types of lesions occurring on the same collection of leaves of the Ootootan variety, those of B being from a larger, rather young, greenish brown spot with a dark brown border and those of C from an older spot with the usual dark brown border and ash-gray or white center. It was at first thought that these two types of lesions might be caused by two different species of *Cercospora*, but the similarity



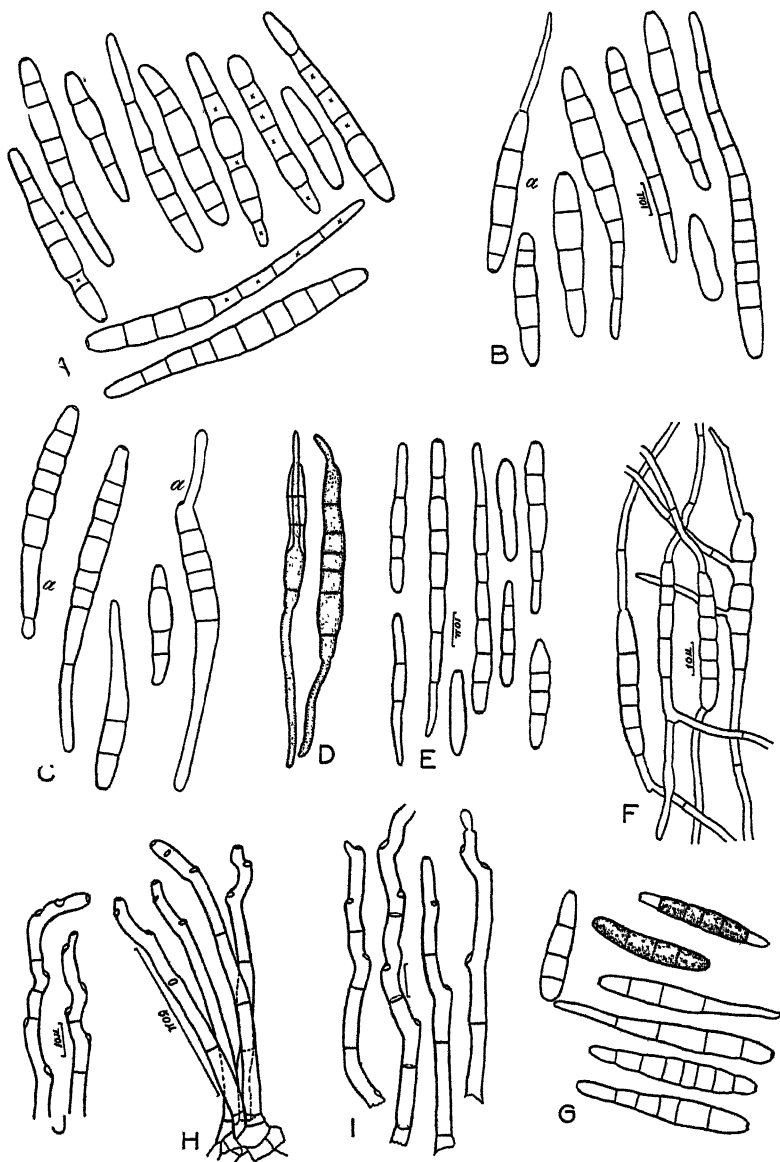


FIG. 8.—Camera lucida drawings of conidia and conidiophores from specimens of frog-eye collected at different times and places

A.—Conidia from leaves of the Biloxi variety of soy bean collected in Pitt County, September 21, 1928. Drawn seven days after date of collection. The cells marked *x* had lost turgidity following disintegration of their protoplasts. Approximately 25 per cent of the conidia of this collection were in this condition. The remaining cells were turgid and capable of germination.

B, C.—Conidia from leaves of the Ootootan variety collected in Perquimans County, September 2, 1928, those of B coming from a large rather young greenish-brown spot with dark-brown border, and those of C from an older spot with the usual ash-gray or white center and dark-brown border. Conidia marked *a* were germinating when drawn.

D.—Germinating conidia scraped from a leaf 81 days after date of collection and 24 hours after the air-dry leaves had been placed in a moist chamber.

E.—Conidia from leaves of the Ootootan variety. Collected October 1, 1925, from the field in Currituck County, in which the disease was originally found in North Carolina.

F.—Conidia of E germinating after 18 hours in tap water.

G.—Conidia from specimens of frog-eye received from Japan.

H, J.—A cluster of conidiophores from leaf of the Biloxi variety.

I, J.—Conidiophores from leaves of the Ootootan variety of different collections, I coming from the collection noted under B and C above, and J from collection noted under E.

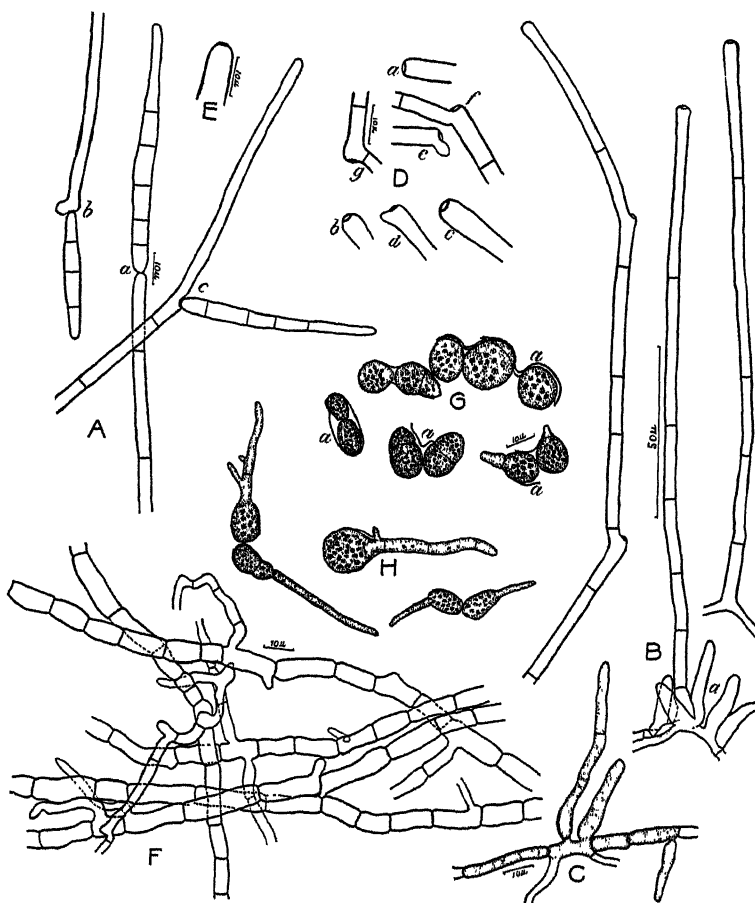


FIG. 9.—Camera lucida drawings showing morphological features of *Cercospora diazi* grown on culture media

A.—Conidiophores drawn with conidia in position: *a*, first conidium fully developed; *b*, a conidiophore beginning proliferation to form a second conidium; *c*, further development.

B.—Conidiophores grown on plain agar. These show numerous septations, prominent geniculations, and brown color. The short hyphae at *a* are young conidiophores. They have already turned brown, while the hyphae from which they arise are still hyaline.

C.—Young brown conidiophores arising from a hyaline hypha.

D.—Higher magnification showing terminal spore scars at *a*, *b*, *c*; continuation of growth beyond the spore scar at *d* and *e*; and geniculations with spore scars at *f* and *g*.

E.—Basal end of a spore showing scar at place of attachment to conidiophore.

F.—Mycelium of a young culture on a synthetic agar medium. Main hyphae are dilute brown, secondary hyphae hyaline.

G.—Swollen hyphal cells, "endospores," characteristic of the older mycelium produced on potato agar having a hydrogen-ion concentration of  $P_{H}$  2.6. The protoplasts have swelled and ruptured the mother-cell wall and have formed new investing membranes. Vestiges of the mother-cell wall are visible at *a*.

H.—Cells of the type shown in G, germinating after 18 hours in tap water.

of the conidia produced on the two kinds of lesions does not warrant such an assumption. The spores of Figure 8, E, were also from Ootootan leaves but from a collection of the previous year in a different part of the State. This collection came from the field in which the first specimens of frog-eye were found in North Carolina but the leaves were gathered 12 days later, at which time the lesions were old and no longer sporulating readily. The spores of this one collection averaged one to two microns narrower than those of collections made at other times and places, and might be considered by some students to represent a different species of *Cercospora*. However, since they are about the same in length as spores found on other collections and the lesions on which they occurred were not different from those of other collections, these spores are not herein treated as representing a separate species. The spores illustrated in Figure 8, G, were obtained from leaves furnished by T. Hemmi. These leaves had been collected in Japan in 1924 and the fungus on them had been identified by Hemmi as *Cercospora diazu*. The conidia on this material were few in number and somewhat shorter than those found on the frog-eye lesions so far collected in North Carolina. However, in diameter, shape, septation, and general appearance the conidia on the Japanese material are much like those found on the North Carolina collections and are here considered to represent the same species. It is generally believed that moisture conditions greatly influence dimensions of the conidia and conidiophores in the genus *Cercospora*. In the present study when pure-culture inoculations were made on soy-bean plants growing in glass-sided incubators where moisture was relatively abundant, both conidiophores and conidia, formed on the lesions which developed, were considerably longer and somewhat slenderer than those found on material collected in the field. (Table 2.) In this respect they were more like conidia and conidiophores grown in culture. Thus it seems that the difference noted above in respect to size of conidia on different collections may be due to growth under different moisture conditions.

TABLE 1.—*Conidia of Cercosporae from soy bean and cowpea distributed in classes on the basis of number of septa per conidium*

Source of conidia	Date and place of collection	Number of conidia in each septation class									Total number of conidia
		1 sep-tate	2 sep-tate	3 sep-tate	4 sep-tate	5 sep-tate	6 sep-tate	7 sep-tate	8 sep-tate	9 sep-tate	
Ootootan.....	October, 1925, Currituck County.	9	11	38	13	15	12	14	0	1	113
Mammoth Yellow.	September, 1926, Pitt County.	7	5	44	12	13	13	9	4	2	109
Biloxi.....	September, 1926, Pitt County.	3	3	33	7	12	8	8	0	1	75
8-day-old culture of <i>C. diazu</i> on plain agar.	-----	3	4	39	11	6	5	2	2	1	73
21-day-old culture of a cowpea <i>Cercospora</i> on potato agar.	-----	1	3	18	15	24	24	14	3	-----	102

TABLE 2.—Average sizes of the conidia falling in the different septation classes, shown in Table 1

Source of conidia	Average size of conidia in each septation class								
	1 septate	2 septate	3 septate	4 septate	5 septate	6 septate	7 septate	8 septate	9 septate
Otootan, 1925, Currituck County.	33.7×4.1	39.8×4.6	43.2×5.3	57.6×5.5	61.2×6.7	64.6×7.1	76.7×6.6	-----	88×8
Mammoth Yellow, 1926, Pitt County.	37.7×6.3	38.4×6.8	42.1×7.2	51.4×7.6	52.7×7.4	59.3×7.6	66.6×7.6	78.5×8.2	88×8.3
Biloxi, 1926, Pitt County.	38.5×5.3	32.3×7	45.8×7.0	48.8×7.0	55.8×6.6	59.2×7.3	67.7×7.7	-----	-----
8-day-old culture of <i>C. diazi</i> on plain agar.	36.6×5.2	79.5×6.2	69.3×7.1	79.1×7.1	80.6×7.6	87.4×7.7	108.7×7.4	92.5×7.8	-----
21-day-old culture of a cowpea <i>Cercospora</i> on potato agar.	-----	42.0×8.2	49.0×7.5	57.2×7.8	61.7×7.8	68.2×7.9	73.8×7.8	77.3×7.7	-----

## GERMINATION OF THE CONIDIA

The conidia germinate readily in tap water by sending out slender hyaline hyphae. (Fig. 8, D, F.) The process begins at favorable temperatures within an hour or so, and by the end of 18 hours long hyphae have been produced. Usually the end cells of a given conidium germinate first, but germ tubes may arise from one or more intervening cells of the same spore. At times two germ tubes arise from a single cell. In cultures conidia often germinate in situ, and have been observed to form a short germ tube which in turn produced a secondary conidium of normal size while the whole was still attached to the conidiophore.

Conidia are still able to germinate after a considerable time in a dry condition. Leaves of the Biloxi variety of soy bean were collected on September 24, brought into the laboratory, and stored in a dry condition in envelopes. On December 2, 69 days after the date of collection, conidia from lesions on these leaves were put in tap water to germinate. After 24 hours at 25° C. approximately 1 per cent of the conidia had germinated, sending out long hyphae. In a similar test conidia from Otootan leaves, which had been collected on August 30 and kept dry in the laboratory for 94 days, showed somewhat less than 1 per cent of germination after 24 hours at 25°. Spores which did germinate produced long vigorous germ tubes. The protoplasm in those spores which failed to germinate had lost all semblance of organization.

Dry leaves from the collections used in the above tests were at the same time placed in the damp atmosphere of a moist chamber. Twenty-four hours later spores were scraped from lesions on these leaves and about 1 per cent were found to have produced short germ tubes. (Fig. 9, D.) Spores from the same packet of leaves had shown nearly 100 per cent germination when tested after 24 days of storage.

Not all the cells of a multiseptate conidium lose viability at the same time. In old spores some of the cells appear to be entirely empty and are much shrunken, while in adjacent cells the protoplasts not only retain their usual finely granular structure but often appear to be more dense than in young conidia. It is not impossible

that this increased density is due to an absorption of protoplasmic materials from the cells which become empty by the cells which remain viable. Moreover, the longevity of the latter cells may be conditioned on this absorption.

#### INOCULATION

A suspension of conidia was obtained by macerating diseased tissue in water. This suspension of conidia and particles of diseased tissue was rubbed onto leaves of Laredo and Mammoth Yellow soy beans growing in the greenhouse. The Laredo plants were young and bore two to three trifoliate leaves; the Mammoth Yellow plants were older and carried many leaves. Inoculations were made in September 2, and the plants were kept under shaded bell jars in the greenhouse for two days thereafter. On September 12, 10 days after the inoculations, small red spots, the first evidence of infection, were found on Laredo, none being observed on Mammoth Yellow at this time. By September 20 the spots on Laredo had become typical frog-eye lesions and bore conidiophores and conidia. Several lesions were also found at this time on Mammoth Yellow leaves. Two days later these bore sporophores and conidia of the frog-eye fungus.

Another inoculation test was made using conidia from pure cultures. The conidia were obtained by gently washing the surface of a 92-day-old culture with tap water. This culture was growing on potato agar in a Petri dish kept in a moist chamber dish in the open laboratory since its inoculation. The colony had by this time reached 1 cm. of the edge of the dish and was producing conidia abundantly at its margins. The conidia were atomized onto soy beans of the Mammoth Yellow variety which had been growing for 22 days in glass-sided incubators at a temperature ranging from 18 to 24° C., but remaining for the greater portion of the time at 18°. At the time of inoculation the plants were tall (averaging 30 cm.) and bore three or four leaves each, but spindling owing to growth in reduced light. The leaves were thinner than normal apparently because of the reduced light and rather high humidity of the culture chambers. They were also smaller than normal for field-grown plants, but possessed a very good color and appeared healthy in every respect.

During the period of infection and development of the disease the temperature of the plant chamber ranged from 18° C. (at night) to 25° (in the afternoons) and averaged about 22°. There was no visible evidence of infection 5 days after inoculation, but at the time of the next observation, 11 days after inoculation, many red-bordered spots were in evidence on the leaves, the largest being about 1.5 mm. and the smallest about 0.5 mm. across. (Fig. 10.) The largest of these lesions must have been evident 8 days after inoculation. On the thirteenth day after inoculation some of the spots had produced sporophores and conidia typical of the fungus used in making the inoculations; also at this time, when the oldest spots were forming their first conidia, young new lesions were appearing in considerable numbers. Obviously the later-appearing lesions arose from conidia which had encountered some difficulty in establishing parasitic relationship with the host. Not only did conidia and conidiophores develop on older lesions but the fungus was recovered in pure culture by planting leaf tissue from young lesions which had shown no fruiting structures. It is judged that the period of incubation for this

disease under favorable moisture conditions and an average daily temperature of about 22° varies from 8 to 14 days.

Several other successful inoculation tests were made, but these need not be reported in detail. Disease lesions resulting from the inoculations made in the greenhouse were comparatively few in number, but they were typical of frog-eye symptomatically and bore fruiting structures identical in every way with those found so abundantly on lesions in the field. Apparently the environmental conditions maintained about the plants in the greenhouse inoculation tests

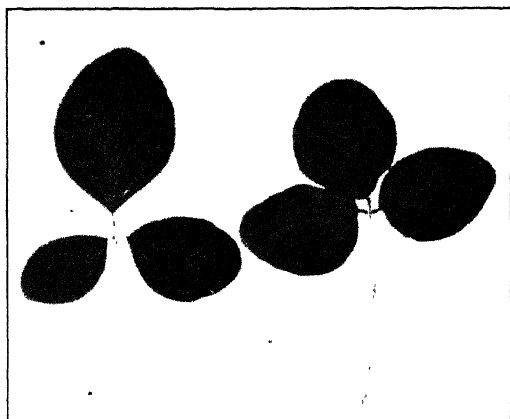


FIG. 10.—Lesions resulting from artificial inoculation of plants of the Mammoth Yellow variety of soy bean. The leaves are smaller and thinner than normal as a result of growth in glass-sided chambers under conditions of rather high humidity and reduced light

were not so favorable for infection as those which occur at times in the field. Greater numbers of lesions developed in the test in the glass-sided plant chambers where humidity was higher and the plants more tender and growing rapidly. Nearly all the infections developed on leaves which were not yet full grown at the time of inoculation, very few lesions appearing at any time on the older and lower leaves. In a test in which young and old plants were inoculated

at the same time from the same culture of the fungus, by far the greater number of lesions developed on the young plants, although these bore fewer leaves with much less total leaf area exposed to infection. Moreover, the average size of mature lesions occurring on young leaves is considerably greater than that of lesions produced on old leaves under the same conditions. Young leaves are not only more susceptible to infection but the fungus grows more rapidly in their tissues. Spores which do not immediately bring about infection of more mature leaves under conditions favorable for development of the disease on young plants sometimes live for several weeks on leaves. Then when conditions are made highly favorable as by covering the plants for a week with a bell jar, these spores grow and produce frog-eye lesions.

#### IDENTITY OF THE CAUSAL FUNGUS

The frog-eye disease of soy bean herein described is in all its major aspects like the leaf-spot disease which Miura (9) found in south Manchuria in 1918 and to which he ascribed, as causal organism, the new fungus *Cercospora diazu*. Likewise, the fungus found by the writer to be associated with frog-eye is morphologically very like that described by Miura. Miura's statement of the symptoms of the disease is very brief. Specimens sent from Japan by Hemmi and from Shizuoka by Nakata have been compared with material

collected in North Carolina, South Carolina, Georgia, and Louisiana and found to be identical in symptomatology and in the morphology of the causal organism. In order that it may be more readily accessible to American pathologists and mycologists, Miura's description is given herewith.

*Cercospora diazu* M. Miura, n. sp.

Amphigenous, spots at first dark brown, later pale brown at centre, irregular or circular in outline, solitary, rarely confluent, with dark colored border, 3-6 mm. across; conidiophores pale sooty colored, springing from a small stroma composed of a few cells, nonseptate or very rarely septate, 100-130 x 5; conidiospores hyaline, cylindrical or fusiform, apex rounded and the base somewhat acute, more or less thicker walled, 0-6 septate, not constricted at septa, aecrogenous, 39-70 x 5-7.

On leaf of *Glycine hispida* Maxim. and *Glycine soja* S. et Z. Tu-men-ling, South Manchuria, August 19, 1918., leg. M. Miura.

The writer has observed certain minor details in which the fungus found in the United States differs from the description given by Miura. The vast majority of the conidia are hyaline, but an occasional sooty-colored spore is found on material kept dry in the laboratory for a few months. These dark-colored conidia have never been observed on leaves fresh from the field. Miura describes the conidia as cylindrical or fusiform and as having a rounded apex and somewhat acute base. As the writer has observed the conidia their greatest thickness is a measure from the base equal to one-fourth or one-third their total length and they taper much more strongly toward their apexes than toward their bases. (Fig. 8, A, B, C, E, and G.) The apexes are usually rounded but are sometimes long and acute. This was observed not only on American specimens but also on material received from Japan. It may be that the acute tip ends of certain of these conidia are the result of prolonged growth somewhat akin to germination. Again, the bases of the conidia can not be described as acute as indicated by Miura, for they are broader and more rounded than the apexes. The writer has also observed a greater range of conidial septation than that given by Miura, and also a greater range in size. However, this is probably merely the result of the observation of a greater number of conidia. Another point of difference in the material observed by the writer is found in the septation of the conidiophores. Septa quite commonly appear in the conidiophores, often occurring between successive geniculations (fig. 8, H, J, and fig. 9, B), but more commonly below the first spore scar (fig. 8, I). This is in marked contrast to the idea conveyed by Miura's words "nonseptate or very rarely septate." On the other hand, the lengths of the conidiophores observed by the writer are not so great as those reported by Miura.

The morphological differences noted above might be used by some students to establish a new species. It appears to the writer, however, that Miura's description of *Cercospora diazu* was based on a rather limited amount of material. Because of agreement in the broad morphological characters of the causal organisms and the very great similarity of the symptom complex resulting from parasitism of the common host, the writer prefers to consider the causal organisms associated with frog-eye as identical with *C. diazu*.

*Cercospora glycines* Cooke (4, p. 39) has been described as the cause of a leaf spot of *Glycines clandestinae* in Australia. This fungus is in

all probability not identical with *C. diazu* on soy bean. The two fungi not only occur on different host species, but differ also in the shape, size, color, and septation of their conidia.

#### PATHOLOGICAL ANATOMY

The exact method of infection, whether through stomatal apertures or by direct penetration of epidermal cells, has not been determined. The germination of conidia sown on leaves in moist chambers has been observed. Germ tubes which develop into long branching hyphae and form a sparse surface mycelium grow out from the conidia. These hyphae have been observed to grow directly across open stomata, but in no instance has it been possible to find a hypha entering the leaf through the stomatal aperture, nor even by direct penetration of the epidermal cells. It is thought that penetration may occur only after the hyphae have grown superficially for some two to four days and killed the epidermal cells by secretion of some toxic substance. Microtome sections of leaves fixed at the time the lesions first become visible show that the epidermal cells have completely collapsed over the entire area of the lesion and in some instances for a short distance beyond the marginal limit of dead mesophyll cells.

Mycelium is not at all abundant in diseased tissues. One usually finds not more than one to three short pieces of hyphae in a stained median section through a lesion. Even in the immediate vicinity of the minute stomata from which the conidiophores arise hyphae are difficult to find. Only a small proportion of the cells of the diseased area are in direct contact with hyphae of the parasite. The fungus apparently secretes some substance which by diffusion passes beyond the frontier of hyphal penetration and injures the host cells. Hyphae may be found near the margins of lesions, but always there is present the reaction indicating injury some two or more cells beyond the most advanced fungus filaments.

The first visible cytological change in injured cells as observed at the margins of sectioned lesions is a slight disintegration of the chloroplasts, accompanied by a change of staining reaction. Usually this is quickly followed by disorganization of the protoplast, loss of turgidity, and eventually by complete collapse of the cell. Apparently much cell-wall material is consumed, for only fragments of the cellulose walls remain in the older portions of the lesions. Epidermal cells are less resistant to toxic action of the fungus and always show complete collapse before the underlying cell protoplasts have lost turgidity. The older diseased areas are shrunken and much thinner than healthy tissue.

As the lesions increase in size the margin is marked by a reddish-brown band of varying width. This band indicates the region in which the cell protoplasts are undergoing disintegration. When the disease has run its course and the infected areas have reached full size, this marginal reddish band of tissue is often found to be thicker than that of adjacent undiseased tissue. (Fig. 7, D.) This thickening of the leaf tissue is due to hypertrophy of the cells delimiting the diseased area. By this time the fungus is exhausting itself in sporulation, and possibly the hypertrophy of host cells is due to stimulation resulting from the presence of very minute traces of the same toxic



material which when present in larger quantities causes cytolysis and death.

Diseased cells which are showing the early disintegration phenomena described above and also those which show hypertrophy always stain more deeply and are much less easily destained than either healthy cells or cells in the older portions of the lesions. The reddish-brown border marks the region of most deeply staining cells.

#### CULTURAL CHARACTERS

*Cercospora diazu* grows well on a variety of culture media in common laboratory use. Unlike many *Cercosporae*, this species sporulates plentifully on all media which support normal even though very sparse growth.

On plain agar (20 gm. chopped agar shreds in 1,000 c. c. of water) thin gray colonies form with a sparse development of aerial hyphae. The colonies are plainly evident at the end of five days and attain a diameter of 8 to 12 mm. in eight days. At this time long smoky-brown conidiophores bearing spores are present. The conidiophores become smoky brown even before forming their initial conidia (fig. 9, B, C), while the conidia and young feeding hyphae remain hyaline. Older hyphae (fig. 9, F) and more mature conidiophores are darker brown and give a gray to brown aspect to the colony, the color becoming darker as the number of conidiophores increases. Plain agar is well suited as a medium on which to study conidiophore development. Growth is not so dense but that individual hyphae can be traced for long distances and conidiophore primordia (fig. 9, B, A, C) easily found.

On potato-dextrose agar<sup>6</sup> the colonies develop as compact mycelial mats with velvet surfaces formed by close stands of vertically growing hyphae. By the time the colony has reached a diameter of 20 to 25 mm. its surface is marked by convolutions or folds disposed in both radial and concentric fashion. (Fig. 7, E.) The radial ridges, some 4 to 10 in number, are the more prominent, the circular folds being plainly evident but less pronounced. The surface of the colony is not only characterized by these physiographic features but is also marked by concentrically arranged color bands. The outermost edge of the colony where the young hyphal tips are invading the medium is a broader circular band of olive green and rearward of this is a circular band of gray more or less tinged with brown. This gray color is due to the ends of white hyphae growing up from the mat beneath. The central and oldest area of the colony surface is usually dark gray to deep olive brown. The marginal contour is not fimbriate but uniformly circular. The reverse of the colony is jet black, and there is no diffusion of color into the substratum.

On a synthetic agar medium (CaNO<sub>3</sub>, 1.22 gm.; KH<sub>2</sub>PO<sub>4</sub>, 2.44 gm.; MgSO<sub>4</sub>, 3.69 gm.; and glucose, 20 gm. in 1 liter of distilled water) the color aspect was much the same as on potato agar, but the mat was not so dense and the diameter of the colony was smaller at the end of the same period. Fewer conidia were present on this medium.

<sup>6</sup> 200 gm. Irish potato, peeled, sliced thin in 1,000 c. c. distilled water. Autoclave 20 minutes at 15 pounds. Strain through cheesecloth, add 25 gm. chopped agar shreds and 20 gm. dextrose. Heat to boiling to melt agar. Adjust reaction to grass green to brom thymol blue. Autoclave. Readjust reaction. Filter through absorbent cotton, tube, autoclave.

The ability of the fungus to grow at different degrees of acidity was tested on potato-dextrose agar made as indicated above. The medium was adjusted to different hydrogen-ion concentrations by the addition of appropriate quantities of normal NaOH or HCl after the final sterilization. The cultures were made in triplicate in Petri dishes poured to a uniform depth of medium, uniformly inoculated, and incubated at 25° C. Colony diameters were recorded daily and taken as the measure of growth. On the alkaline side of neutrality growth occurred at the highest hydroxyl-ion concentration used, namely, that corresponding to a  $P_H$  of 9.6. At  $P_H$  8.0, 8.5, 9.0, and 9.6 growth was slow in starting and at the two last-mentioned indexes proceeded at a markedly slower rate than on more acid media. At  $P_H$  8.0 and 8.5, after the tardy start, growth continued at approximately the same rate as on less alkaline media. Growth was initiated most promptly, and continued at the highest rate in the range represented by  $P_H$  3.6, 4.5, 5.5, and 6.9. No growth occurred at  $P_H$  1.6. At  $P_H$  3.6 the colonies reached a diameter of 75 mm. in 44 days and had the usual olive-green to olive-brown velvety surface composed of numerous short aerial hyphae. At  $P_H$  2.6 growth was slow in starting, proceeded at a reduced rate, and produced colonies so abnormal in structure and appearance that they seemed to belong to an entirely different fungus. The colonies were small, having attained a diameter of only 13 mm. in 44 days, and had whitish or cream-colored pustulate or pimply surfaces with little or no suggestion of the usual dark-colored velvety aspect.

When the mycelium of these abnormal colonies is studied microscopically, it is seen, when young, to be composed of very short closely septate hyphae, and, when older, to consist of broken hyphal remnants whose cells have become greatly swollen. Apparently unable at the high acidity to function in normal-growth processes, the protoplasts within the original hyphal cells swell greatly, often bursting the mother-cell wall and each at the same time investing itself with a new thin hyaline wall. Remnants of the old mother-cell wall are frequently clearly visible clinging to the sides of the newly formed cells. (Fig. 9, G.) In swelling, the new cells become elliptical, oval, or spherical in shape, reach a diameter of 2 to 4 times that of normal hyphae, and often form septa which may or may not divide them into equal parts. The swelling is not accompanied by development of large vacuoles as if the energy of the cell were being exhausted in the mere process of enlargement, but the swollen cells are densely filled with protoplasm containing many coarser lumps or granules heaped in the center of each cell. These coarser lumps probably constitute a reserve of nutritive material. This phenomenon is suggestive both of chlamydospore formation in certain fungi and of the type of endogenous spore formation which occurs in the conidiophores of *Thielavia basicola* (1) and *Ceratostomella fimbriatum* (6). The process is not at all similar to budding such as occurs in germination of yeasts and certain other fungi. When these abnormally shaped and enlarged cells are placed in tap water, they germinate in the normal manner characteristic of conidia—i. e., by the formation of slender hyaline germ tubes (fig. 9, H), and when placed on less acid potato agar, they produce colonies of normal structure and appearance.

## DISSEMINATION AND CONTROL

Laboratory tests have not yet been made to determine if the fungus can be isolated from seeds, nor has the disease yet been recognized on pods. However, field observations indicate that the disease is seed-borne. In mid-season observations made on variety tests, the disease has been observed on rows planted to certain varieties while adjacent rows of a different variety, although known to be susceptible, showed no disease until much later in the season. Had inoculation occurred as a result of a wind-borne spore shower from some unknown source, all susceptible kinds in a variety planting should become diseased at approximately the same time. As shown above, spores of the fungus may remain viable as long as 94 days when kept on dry leaves in the laboratory. It is not improbable that in the open field the fungus in leaf and stem lesions remains viable throughout the winter months. The fungus grows readily and sporulates abundantly on culture media and sterilized soy-bean stems. This would indicate that the fungus can grow saprophytically on dead soy-bean tissue and sporulate thereon in the following summer, thus creating a source of inoculum for the new crop. It is therefore recommended that all refuse from a diseased crop be plowed under in order to promote early decay as a means of reducing the amount of inoculum which may overwinter. In addition to this destruction of diseased material, the rotational practice should be such as to allow an interval of one full growing season between successive soy-bean crops on infested fields. In badly infected areas the earlier maturing, more resistant varieties of soy bean should be substituted for the late maturing and more susceptible varieties.

## SUMMARY

The disease of soy bean herein described is designated "frog-eye leaf-spot."

The first American collection to which frog-eye may definitely be referred was made in Louisiana in August, 1925, and in North Carolina in September, 1925. It seems probable, however, that the same disease was actually seen by Moore in South Carolina in 1924.

Lesions occur chiefly on the foliage but have also been found on the stems.

The chief symptoms on leaves are rounded or angled necrotic lesions which are at first redish-brown but later have light brown to ash gray or even white centers, delimited by a narrow reddish-brown border. In old lesions the central tissues are often very thin and the border is often thicker than the surrounding undiseased tissue.

On stems the younger lesions have red centers bordered by a zone of black. Older lesions have pale smoke-gray centers surrounded by a narrow band of red and this in turn by an outer bordering band of black.

The disease is known to be present in at least five States, all of which are in the South. In North Carolina it is widely distributed over the piedmont and coastal plain areas.

Plants are frequently seen on which 25 per cent or more of the total leaf area is diseased. The disease is potentially capable of causing great reductions in yield of seed and quality of hay.

Greatest injury is done to late-maturing varieties such as Ootootan and Biloxi. Early-maturing varieties such as Dixie, Manchu, and Virginia, although susceptible, escape serious injury.

The causal organism is readily isolated, grows well, and sporulates freely on many artificial substrata. The pathogene is described in detail in the body of this paper.

Conidia germinate readily in tap water, and a small proportion of them were still viable 94 days after collection, during which time they were kept on dry leaves in the laboratory.

Successful inoculations with conidia from pure cultures and the subsequent recovery of the same organism from lesions produced by such inoculations establish the pathogenic causal relation of the organism commonly associated with the disease.

The causal fungus is identified as *Cercospora diazu* Miura, first collected and described by Miura from south Manchuria in 1918. The fungus observed in North Carolina differs in certain minor morphological details from the description given by Miura, but it is held that these differences are not sufficiently pronounced nor yet sufficiently well fixed to justify the establishment of a new species for the organism present in America.

The fungus causes pathological changes in the host by means of some substance which acts in advance of the foremost hyphae. The host cells first show change in staining reaction. This is followed by protoplasmic disorganization and complete collapse of affected cells, only fragments of the cellulose walls remaining in the older portions of the lesions.

On potato-dextrose agar the causal fungus forms colonies characterized chiefly by a combination of prominent radial folds and less evident circular convolutions accompanied by concentrically arranged color bands of white, olive green, olive brown, and gray. Growth occurs over a range of acidity extending from  $P_H$  2.6 to 9.6 or beyond. At  $P_H$  2.6 growth is very slow and results in the formation of morphologically abnormal mycelium.

It is believed that the fungus may overwinter on diseased leaves and stems and on seed from diseased fields. The value of seed treatment has not yet been established. Control measures believed to be effective are (1) plowing under to promote early decay of infective material, (2) a rotation of two or more years, and (3) use of earlier and more resistant kinds of soy beans in place of late-maturing and more susceptible varieties.

#### LITERATURE CITED

- (1) BRIERLEY, W. B.  
1915. THE "ENDONIDIA" OF *THIELAVIA BASICOLA*, ZOFF. Ann. Bot. [London] 29: [483]-493, illus.
- (2) CARVER, G. W.  
1901. SOME *CERCOSPORAE* OF MACON COUNTY, ALABAMA. Tuskegee Norm. and Indus. Inst. Expt. Sta. Bul. 4, 8 p.
- (3) COERPER, F. M.  
1919. BACTERIAL BLIGHT OF SOYBEAN. Jour. Agr. Research 18: 179-194, illus.
- (4) COOKE, M. C.  
1892. AUSTRALIAN FUNGI. Grevillea 21: 35-39. (Also in Saccardo, P. A., Sylloge Fungorum, v. 11, p. 626, Patavii. 1895.)
- (5) HEDGES, F.  
1925. A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EPS. Jour. Agr. Research (1924) 29: 229-251, illus.

- 
- (6) LEHMAN, S. G.  
1918. CONIDIAL FORMATION IN *SPHAERONEMA FIMBRIATUM*. *Mycologia* 10: 155-163, illus.
- (7) ——— and WOLF, F. A.  
1924. A NEW DOWNY MILDEW ON SOY BEANS. *Jour. Elisha Mitchell Sci. Soc.* 39: 164-169, illus.
- (8) MATSUMOTO, T., and TOMOYASU, R.  
1925. STUDIES ON PURPLE SPECK OF SOYBEAN SEED. *Ann. Phytopath. Soc. Japan* 1 (6): 1-14, illus. (Abstract in *Rev. Appl. Mycol.* 4: 714-715, 1925, and in *Japan. Jour. Bot.* 3 (1): (12-13), 1926.)
- (9) MIURA, M.  
1921. [DISEASES OF THE MAIN AGRICULTURAL CROPS OF MANCHURIA.] *So. Manchuria Ry. Co. Agr. Expt. Sta. Bul.* 11, 56 p., illus. [In Japanese. English abstract in *Japan. Jour. Bot.* 1 (1): (9), 1922.]
- (10) RIDGWAY, R.  
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.
- (11) WOLF, F. A.  
1920. BACTERIAL BLIGHT OF SOYBEAN. *Phytopathology* 10: [119]-132, illus.
- (12) ———  
1925. BACTERIAL PUSTULE OF SOYBEAN. *Jour. Agr. Research* (1924) 29: 57-68, illus.
- (13) ——— and LEHMAN, S. G.  
1926. BROWN-SPOT DISEASE OF SOY BEAN. *Jour. Agr. Research* 33: 365-374, illus.
- (14) ——— and LEHMAN, S. G.  
1926. DISEASES OF SOY BEANS WHICH OCCUR BOTH IN NORTH CAROLINA AND THE ORIENT. *Jour. Agr. Research* 33: 391-396.



2

## WITCHES' BROOM OF POTATOES AND TOMATOES<sup>1</sup>

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### POTATO WITCHES' BROOM

#### INTRODUCTION

A study of potato witches' broom was started at the Montana Agricultural Experiment Station in 1915. At that time it was not

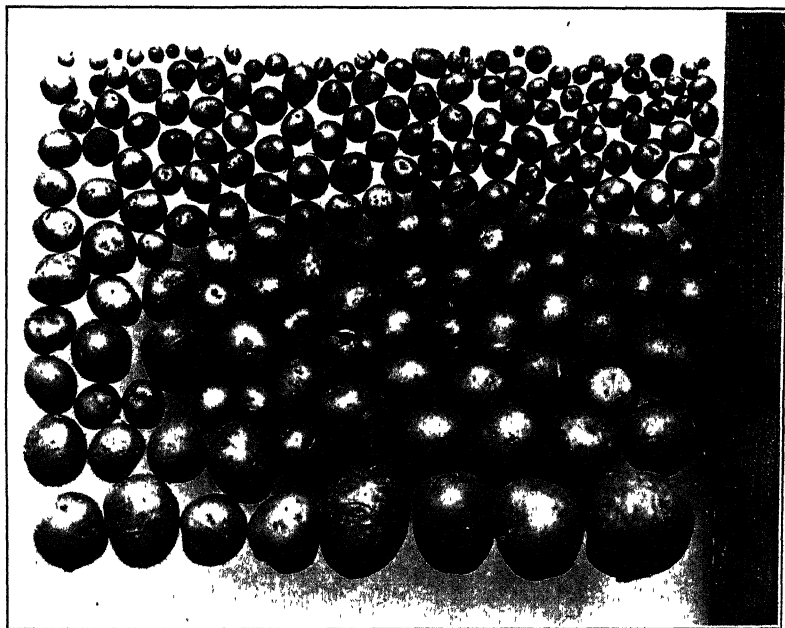


FIG. 1.—Tubers from one potato plant dug September 25, 1915, showing the effect of witches' broom. The 184 tubers cover an area of only 192 sq. cm.

recognized as distinct from the disease caused by *Rhizoctonia*, and was grown experimentally in connection with the latter disease. However, the photographs and descriptions prepared in 1915 and 1916 show unquestionably that some of the potatoes concerned were affected with witches' broom instead of *Rhizoctonia*. (Fig. 1.) The

<sup>1</sup> Received for publication Mar. 27, 1928; issued July, 1928. Published with the approval of the Director of the Montana Agricultural Experiment Station.

<sup>2</sup> Thanks are due Prof. D. B. Swingle for criticizing the manuscript.

records show that this virosis<sup>3</sup> was transmitted by the tubers. Observations on witches' broom were made from 1915 to 1920, but were thereafter discontinued because the disease was not very important economically. Increased prevalence led to intensive study of witches' broom from 1925 to 1928.

In 1925, a strain of Jersey Peachblow potato was discarded because 10 per cent of the plants had witches' broom. The next year, in a sample of a stock of Bliss Triumph potatoes submitted for certification, 57 per cent of the 98 hills in the test row showed disease. The tubers used in planting this row weighed from 4 to 8 ounces apiece, and were cut into about 2-ounce seed pieces. The disease recurred in serious amounts in 1927 when a 6-acre field of Bliss Triumph potatoes grown from certified seed at Three Forks, Mont., showed 17 per cent of witches' broom. Many other fields likewise showed increases in witches' broom since 1925, but the amount of the disease in those cases was less than 17 per cent of the crop. In the State of Washington the ravages of witches' broom destroyed a 30-acre field of potatoes, there being 100 per cent infection.<sup>4</sup> Jaczewski (9)<sup>5</sup> reports this disease in Russia.

Observations and experiments have not yet revealed the method by which the disease spreads in the field. It occurs in the progeny of potatoes which show no symptoms easily recognizable by men roguing the fields. This virosis is very injurious to potatoes, for affected plants produce very few marketable tubers even when they show only primary symptoms.

#### SYMPTOMS OF POTATO WITCHES' BROOM

Potato witches' broom was well illustrated and described by Hungerford and Dana (8). It was illustrated and briefly described by Whipple (23), Coons and Kotila (3), Bisby and Tolaas (2), McKay (15), and Sanford (19). Descriptions of witches' broom were also given by Young and Morris,<sup>6</sup> Young (24), McLarty (17), and Cutler and Sanford (4). Spindling sprout is unlike witches' broom in that it is not a single disease due to a single cause, but is just a symptom caused by physiological conditions, leaf roll, or witches' broom.

The primary and secondary symptoms of witches' broom are usually distinct, although they intergrade frequently. The first symptom is an increasingly prominent flavescence<sup>7</sup> in the new leaflets on one or more stems. Marginal flavescence, which consists of light green or yellow margins on green leaves, was observed in 10

<sup>3</sup> "Virosis" is a name for virus disease proposed by Dr. L. R. Jones at Lincoln, Nebr., on Dec. 28, 1925.

<sup>4</sup> UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. WITCHES' BROOM OF POTATO. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 10: 72. 1926. [Mimeographed.]

<sup>5</sup> Reference is made by number (italic) to "Literature cited," p. 853.

<sup>6</sup> YOUNG, P. A., and MORRIS, H. E. POTATO WITCHES' BROOM IS A TRANSMISSIBLE DISEASE. (A PRELIMINARY REPORT.) U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 10: 26-28. 1926. [Mimeographed.]

<sup>7</sup> The term "flavescence" is proposed to describe the abnormal, nonnecrotic, hyaline appearance and light green or yellow color that characterize the leaves and young stems of diseased plants, at least during the developmental stages preceding maturity. "Chlorosis" is a broad general term commonly used to describe the light green or yellow color noted in diseased plants, and there seems to be no possibility of limiting its meaning to any particular category, even to the extent of separating it as a nonnecrotic symptom from yellowing. Schultz and Folsom (20) give a common definition of chlorosis. The term "flavescence" is useful in describing the particular kind of chlorosis occurring in witches' broom, mosaic mottling, peach yellows, and other diseases in which chlorophyll development is abnormally diminished in the juvenile stages. Another kind of chlorosis is the interveinal chlorosis or yellowing of potato leaf roll. Leaves with normal green color slowly become yellow to greenish hyaline between the veins. This is a slow necrosis preceding death by a few weeks. Young and Morris (9) described this interveinal yellowing.



varieties of potatoes affected with this disease. Bright yellow margins were seen in the senescent leaves of one normal potato, but this was unlike the marginal flavescence due to witches' broom. Appel (1) mentions a marginal yellowing of potato leaves, but his colored plate shows no symptom closely resembling the marginal flavescence caused by witches' broom.

#### PRIMARY SYMPTOMS

The tops of potatoes showing the first symptoms of witches' broom grow rapidly, producing new leaflets that are dwarfed, flavescent, and often rugose. (Figs. 2, 3.) The stems bearing them are also flavescent and have unusually long internodes and enlarged nodes. Such stems are cylindrical rather than quadrangular and winged, and even the leaf petioles have wings of less than usual prominence. The lower leaves are normal on potatoes with primary witches' broom because these developed before the appearance of the disease. (Fig. 2.) Especially in the field the tops of witches'-broom potatoes often become purple, and the flavescent margins of the leaflets frequently become pink or red. Whipple (23) illustrated a potato vine with primary witches' broom, although he called it a "yellow top degenerate." As now understood, "yellow top degenerate" appears to consist of rugose mosaic, witches' broom, or a combination of the two diseases.

Many flavescent, spindling axillary branches develop all along the stems and bear tops with typical symptoms. Also, basal branches arise and the plant soon assumes a bushy appearance. (Fig. 2.)

The tops of witches'-broom plants often bloom and produce fruit in abnormal profusion. Late in the growing season the subterranean tubers sprout and send up very many spindling little stems with dwarfed leaves around the base of the main stem. These tubers sometimes proliferate and thus produce chains of a few small tubers. Many little aerial tubers with leafy eyes commonly develop on the main stems. Plants showing primary symptoms usually bear 25 to 200 very small subterranean tubers. (Fig. 1.) Besides these, they



FIG. 2.—Green Mountain potato grown in the greenhouse, showing the willowy growth, dwarfed leaves, and other primary symptoms of witches' broom transmitted to it by a tuber core from a Bliss Triumph potato. The large leaves were produced before the disease symptoms appeared.  $\times \frac{1}{4}$

generally bear 1 to 3 average-size tubers that developed before the disease symptoms appeared.

Frost usually kills the plants in the field near this stage in the progressive appearance of the symptoms. Their continued development was studied in the greenhouse in plants manifesting primary symptoms as the result of stem grafts and tuber-core grafts. The secondary symptoms appear and gradually replace the primary symptoms. Many of the primary and secondary symptoms are identical and often intergrade.

The primary symptoms of witches' broom are the same in the greenhouse and the field, except that greenhouse plants frequently become



FIG. 3.—Bliss Triumph potato showing primary symptoms of witches' broom transmitted to it by a tuber core from a Jersey Peachblow potato tuber. The top shows many spindling branches with dwarfed, flavescent leaves. The base bears spindling sprouts.  $\times \frac{1}{7}$

very tall and willowy, with narrow vines that are often 0.8 to 1.2 meters tall, while field plants are broad and bushy, or small and upright, depending upon the severity of the disease. (Figs. 2, 3.) The uprightness may be due to mosaic in combination with witches' broom.

An unusual type of floral development occurred in the greenhouse in 1926. Aerial tubers appeared on the old flower trusses of two Bliss Triumph potatoes, and new flowers grew from the eyes of the tubers. (Fig. 4.) One of these plants showed primary symptoms and the other secondary symptoms of witches' broom. Apparently these tubers on old flower stalks possessed the genetic ability to produce flowers because of their position. Large Bliss Triumph plants are ornamental with their yellow to purple tops bearing flowers, red aerial tubers, and filamentous stems. The colors are often brilliant.

## SECONDARY SYMPTOMS

In the further development of a witches'-broom plant, the basal sprouts grow slowly while the main stems grow rapidly. The old leaflets produced while the plant was normal gradually die and are replaced by the dwarfed, chlorotic, often simple leaves produced by the spindling tops and branches. The main stems finally die, completing the transformation of a large, normal potato plant into a dwarfed plant with secondary symptoms. This illustrates the intergradation of the primary and secondary symptoms. (Fig. 2.) While large plants with witches' broom usually bear numerous tubers, plants extremely dwarfed by this disease often produce very few.



FIG. 4.—Flowers produced by the eyes of aerial tubers borne on an old flower truss. Severe witches' broom was transmitted to this Bliss Triumph potato plant by a tuber core from a diseased Jersey Peachblow potato.  $\times 1\frac{1}{2}$

Pubescent, usually leafless, filamentous branches one-fourth to 2 mm. in diameter and one-half to 10 cm. long often appear at many of the nodes of the stem. (Fig. 5.) While this symptom is frequently absent on witches'-broom plants, especially in the field, it is very useful in diagnosis when it occurs. These slender, even threadlike aerial stems are often branched and bear terminal tubers and simple leaves. Sometimes the tips of the filamentous aerial stems grow into the surface of the soil and produce terminal tubers there. Some large plants in the greenhouse bore a hundred or more filamentous stems. Witches'-broom plants in the field, particularly those in cages, often bear such stems. The filamentous stem shown in Figure 5 is unusually elaborate.

On normal Bliss Triumph potatoes in the greenhouse were seen 25 examples of an unusual structure comparable to these filamentous stems. These potatoes bore basal, plagiotropic, hyaline, pubescent,

leafless, apparently functionless stems (or aerial stolons) which were 2 to 12 cm. long and one-half cm. thick. They did not develop further, and so were quite unlike the filamentous stems on diseased potatoes.

While plants that grow from tubers with witches' broom usually display only secondary symptoms, primary symptoms may occur in mild cases of the disease. This was exhibited in the field in 1927 when Bliss Triumph selection No. 115 produced large vines with large compound leaves, even though the plants in the two preceding generations showed secondary symptoms of witches' broom. Very little flavescence occurred in the tops of the vines during most of the season, so the disease was readily detected only by examining the bases of the plants. Each had about 50 stems with some fla-

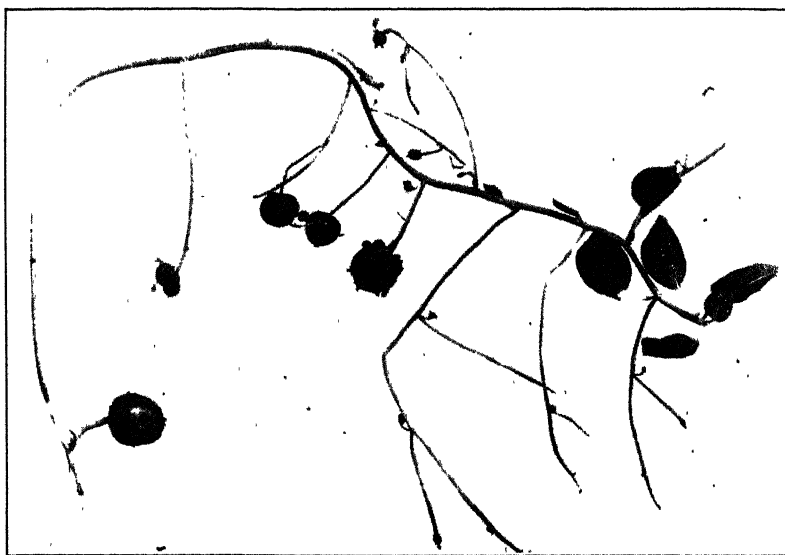


Fig. 5.—Elaborate filamentous aerial stems of a Bliss Triumph potato showing secondary symptoms of witches' broom. The simple leaves are terminal.  $\times \frac{1}{16}$

mentous stems and aerial tubers. These plants had the disease in an unusually mild form, yet they produced no tubers of marketable size. The other potatoes of different parentage showed the disease in more severe form with fewer primary symptoms. Although the strains of potatoes selected for study of witches' broom had the disease in widely different degrees of severity, there is not sufficient evidence to conclude that more than one virus was involved.

Sprouts from tubers produced by witches'-broom plants show symptoms of the disease soon after they appear, for each seed piece sends out 3 to 50 spindling little stems with dwarfed and often simple leaves. (Figs. 6, 7.) This spindling sprout is a characteristic symptom at this stage of the disease. These sprouts usually produce field plants with many dwarfed, spindling, partly procumbent stems 12 to 30 cm. tall. This prostrate form is now known to be the most common type of witches' broom, and has been observed in Montana nearly every year since 1915. (Fig. 6.) Hills of this type were

secured by planting two aerial tubers from the top of one Jersey Peachblow plant. Some of the witches'-broom potatoes in the greenhouse exhibited part of the following unusual symptoms. Some had green vines that were abnormal only in that they were



FIG. 6.—A young Bliss Triumph potato plant with witches' broom, showing numerous spindling stems with small leaves.  $\times \frac{1}{4}$

very spindling and dwarfed. Usually the stems were numerous and dwarfing was extreme. Some potted plants were only 5 to 15 cm. tall at the age of 5 months; most of their leaves were simple. The stems on the smallest of these plants were only one-half to 1 mm. in

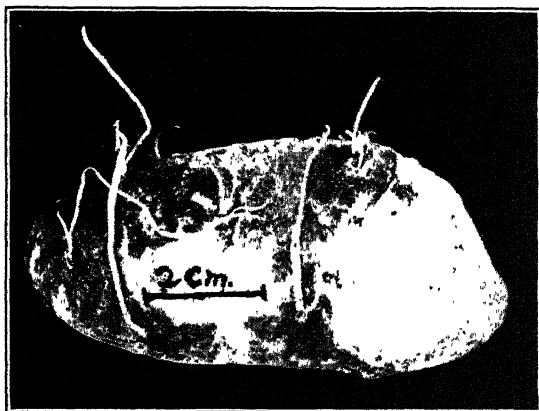


FIG. 7.—Spindling sprouts on a Russet Burbank potato tuber affected with witches' broom. The virus of this disease was transmitted to its parent by an inarch graft with a witches'-broom tomato vine

diameter, and the densely clustered, entirely simple leaves were only 1 to 4 mm. wide. (Pl. 1, F.) On some old witches'-broom potatoes many of the aerial tubers produced 5 to 15 spindling little stems apiece, so that the main stems were adorned with rosettes of little stems. (Fig. 8.) Some senescent vines produced new branches in their tops.

The lower leaves of old witches'-broom plants were sometimes rolled and brittle, suggesting leaf roll. However, they had none of the other symptoms of leaf roll.

Crinkle mosaic often occurs in combination with witches' broom. The symptoms of each disease are usually visible at the same time. In inoculations with both the mosaic and the witches'-broom viruses, the symptoms of mosaic appear first. New leaves produced later which show symptoms of witches' broom often are not mottled.

Tubers produced by witches'-broom plants have a short dormancy period or none, and senescence occurs later in diseased than in normal potatoes. With good care a witches'-broom plant may appear to remain alive for a year or more, as the old stems successively die and

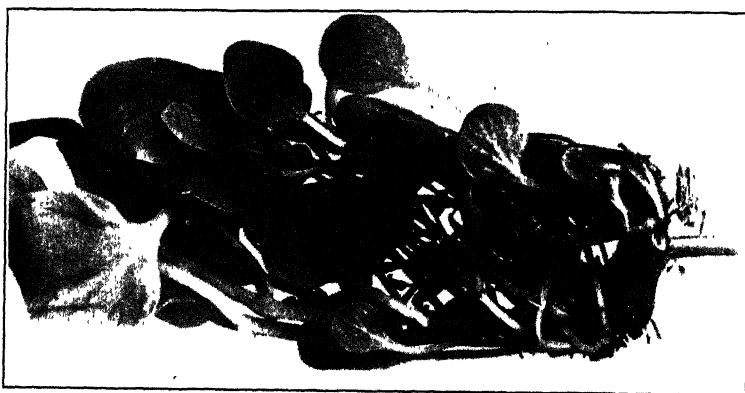


FIG. 8—Bliss Triumph potato with severe witches' broom, showing an aerial tuber bearing a rosette of many spindling stems and simple little leaves.  $\times 1\frac{1}{2}$

are replaced by new sprouts which arise from tubers in or near the surface of the soil.

#### METHODS OF TRANSMITTING WITCHES' BROOM

##### GENERAL METHODS

Seed tubers were cut into 2 to 8 pieces, 1 or 2 of which were saved for controls. The cutting knives and nickel-plated cork borers were disinfected with 5 per cent formaldehyde and then rinsed with water before changing to different scion and stock tubers. The hands were disinfected frequently with laundry soap or a 4 per cent formaldehyde solution. The table was washed with 1 per cent Uspulun before fresh groups of cut seed pieces were laid upon it. Hence, there was little danger of unintentional transference of the witches'-broom virus, especially since it is difficult to transmit. The greenhouse was frequently fumigated with HCN and sprayed with strong nicotine sulphate to control aphids and other insects.

##### CORE GRAFTS

The method of grafting tubers was essentially the same as that described by Murphy and M'Kay (18) and Goss (6). From tubers produced by witches'-broom potatoes, cores 2 to 4 cm. long and 0.5 to 1 cm. in diameter were removed. The small size of the diseased tubers did not permit the use of larger cores. When possible, holes

in the stock seed pieces were made parallel to the vascular rings with cork borers one or two sizes smaller than the ones used in removing the scion cores. The bark was cut from the ends of the scion cores, so that these cores from the witches'-broom tubers certainly bore no eyes. After the insertion of the cores the controls and inoculated seed pieces were usually disinfected with 10 per cent Semesan Bel suspension to lessen subsequent rotting. The seed pieces were then planted in a sand bed. Sometimes seed pieces were kept in a damp chamber for a day after cutting to aid in callusing, but rotting was not diminished by this method. Potatoes were transplanted to 15 or 20 cm. pots as soon as the sprouts were 2 to 6 cm. long. The serial numbers written in India ink on the seed pieces usually were readable at the time of transplanting.

#### STEM GRAFTS

Three methods of grafting herbaceous stems were used: (1) Cleft grafts were made by inserting scions into clefts made in the stocks; (2) slips (scions) were inserted into slits made in the stems of the stocks; (3) inarch grafts were made by slicing off the cortical layers on one side of each of two stems and binding the cut surfaces together while the roots of both plants remained undisturbed in the soil. All grafts were tightly wrapped with string and painted with hot grafting wax. The grafting wax, preferably just hot enough to be liquid, did not injure the stems of the solanaceous plants used. The drops of grafting wax that fell on the leaves slowly caused unimportant leaf spots. This is a rapid method of protecting cut surfaces from injury due to evaporation. High humidity was maintained in the room only while the grafts were being made. Grafts were not unwrapped for one to two months, so growth kept the cut surfaces in close contact. The resulting constrictions of the stems did not appear to be injurious.

#### LEAF-MUTILATION INOCULATIONS

The method of making leaf-mutilation inoculations was similar to that used by Schultz and Folsom (20). Stems and leaves of witches'-broom potato plants were ground in a sterile food grinder and placed in sterile dishes. Care was taken to prevent contamination of the inoculum from the possible presence of a virus in inoculated plants. Each inoculation was made by placing some of the freshly macerated inoculum on a leaf and pressing it against the leaf until the latter was ruptured. Usually 20 of these inoculations were made on each plant. Plants were 8 to 20 cm. tall and growing vigorously at the time of inoculation. In many cases the plants were reinoculated two or three times at intervals of three to seven days. Plants were usually kept damp for 10 to 20 hours after inoculation by spraying water on the steam pipes, walls, and floor more often and more abundantly than usual.

#### EXPERIMENTS IN TRANSMISSION OF WITCHES' BROOM FROM DISEASED TO HEALTHY POTATOES

Witches' broom was transmitted by tuber-core grafts to 59 potato plants of 11 varieties. (Fig. 9.) The incubation periods varied from 29 to 114 days.

The symptom of marginal flavescence was seen in all the affected varieties except Russet Burbank. In other experiments Smooth Burbank potatoes showed this symptom. Similar core grafts failed to transmit witches' broom to Norwegian Yam, Early Michigan, Early Rose, Producer, and Up-to-date potatoes. However, there were too few inoculations of these varieties to indicate immunity.

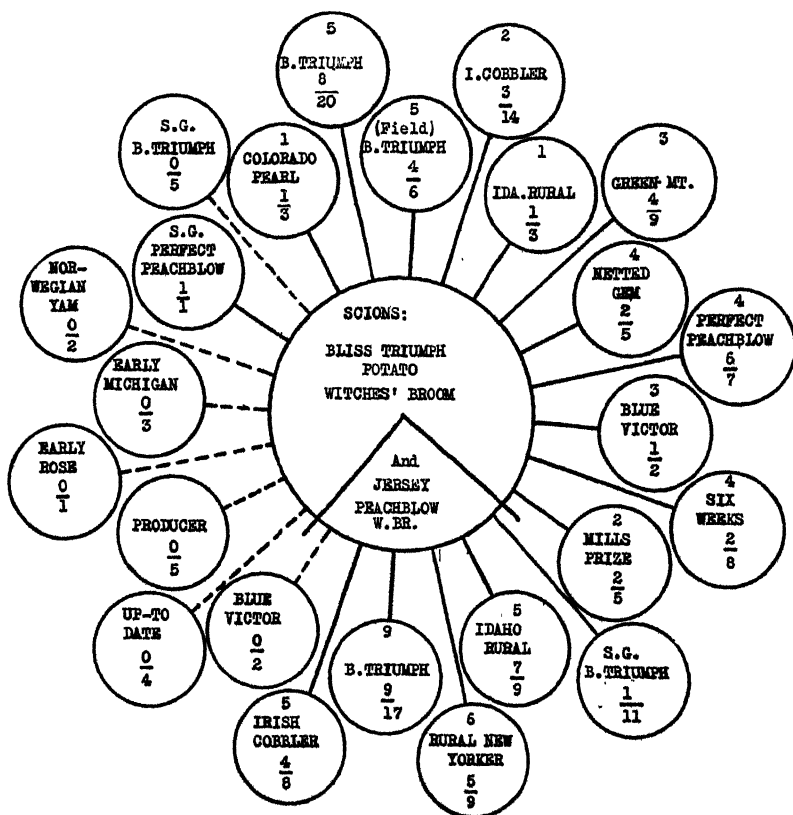


FIG. 9.—Chart showing the transmission of potato witches' broom from 4 selections of Bliss Triumph and 4 selections of Jersey Peachblow potatoes having the disease to 11 varieties of potatoes. Solid lines connecting circles show that infection occurred; broken lines show that infection did not occur. The numbers at the top of the small circles refer to the numbers of healthy controls from the same tubers as those inoculated. In the fractions the denominators represent the number of inoculations and the numerators represent the number of infections. All the inoculations were made by means of core grafts except the three marked "S. G." meaning stem grafts. Twelve of the core-grafted plants, including the four marked "field," were grown in the field. All the others were grown in the greenhouse.

Of the 142 seed pieces core grafted with scions from Bliss Triumph and Jersey Peachblow potatoes containing the witches'-broom virus, 42 per cent developed plants with unmistakable symptoms of the disease. These graft inoculations were made in three series during 15 months. Cutting off the tips of the potato stems before they became senescent stimulated new growth which showed the disease symptoms. This method hastened the appearance of symptoms in inoculated plants. In two cases the disease symptoms did not appear except in the progeny of the inoculated plants. Some pre-



liminary studies on transmission were reported by Young and Morris<sup>8</sup> and Young (24).

Control seed pieces were cut from the same tubers as those furnishing the core-grafted seed pieces. None of the resulting 46 control plants growing near the inoculated plants in the greenhouse developed symptoms of witches' broom.

To study primary symptoms in the field, 30 of the core-grafted seed pieces, with their controls were planted in the field in 1927. Here, the primary symptoms of witches' broom appeared clearly in 4 of the inoculated plants of each of the following varieties: Bliss Triumph, Idaho Rural, and Irish Cobbler. Five of the other 18 core-grafted plants developed questionable symptoms of witches' broom. The 14 field controls remained healthy.

In another experiment 45 normal seed pieces of the Sharples strain of Bliss Triumph potatoes were core grafted with scions from healthy tubers of this variety in an attempt to detect differences between the early and late maturing forms. Cores from the tubers of the early maturing strain were placed in the stock seed pieces of the late maturing strain and vice versa. These plugged seed pieces, and 45 controls from the same tubers, produced plants which grew in the greenhouse for 84 days. None of them developed symptoms of viroses. Besides these control grafts, 4 Bliss Triumph seed pieces were core grafted with scions from healthy Irish Cobbler and Russet Burbank tubers, and 3 Russet Burbank and 2 Irish Cobbler seed pieces were core grafted with scions from healthy Bliss Triumph tubers. None of the seed pieces or the controls from the same tubers developed plants with disease symptoms. These served as checks on the grafts containing diseased scions.

About 10,000 potato plants selected for freedom from witches' broom were closely observed while they developed to maturity in the greenhouse and the field. The parents of these plants had shown no evidence of witches' broom. Only one of these progeny plants developed witches'-broom symptoms, and it probably represented late-season infection of the parent in the field. In contrast to this, hundreds of tubers from witches'-broom potatoes were planted in the field and in the greenhouse, and all of them showed secondary symptoms of the disease, proving that tuber transmission is a regular occurrence.

Vines of 17 normal Bliss Triumph and Perfect Peachblow potatoes were grafted with stem scions from witches'-broom potatoes. Two were inarch grafts and the others were slip grafts. One plant of each variety developed witches'-broom symptoms; one was an inarch graft and the other was a slip graft. Slip grafts were inefficient in transmitting witches' broom under the conditions prevailing, probably because the slips died too soon. Güssow (?) transferred mosaic by inarch grafting.

One normal Russet Burbank and four normal Bliss Triumph potatoes were inarch grafted on witches'-broom tomato plants. The tomatoes had acquired the disease through stem grafts with witches'-broom potatoes. One potato of each variety developed vine and tuber symptoms of witches' broom, showing the transference of the virus back to potatoes. (Fig. 7.) In six inarch grafts of

<sup>8</sup> YOUNG, P. A., and MORRIS, H. E. Op. cit.

healthy Bonny Best tomatoes on Russet Burbank potatoes free from witches' broom, none of the plants were affected by the grafts.

By leaf-mutilation inoculations 71 Bliss Triumph, Russet Burbank, and Irish Cobbler potato plants 12 to 30 cm. tall were inoculated with the witches'-broom virus. These experiments were conducted in the greenhouse in three series during two years. None of the inoculated plants or controls developed witches'-broom symptoms. Tubers from 36 of these inoculated plants were grown later in the greenhouse, but none of them developed witches'-broom symptoms. Although rugose mosaic was transmitted frequently by leaf-mutilation inoculations made at the same time, this method appears to be ineffective in transmitting witches' broom.

Eighteen normal potato plants were grown in pots with plants having secondary symptoms of witches' broom, but none of the normal plants became diseased. No evidence of transmission of witches' broom by soil or by root contact was seen by the authors.

Mealy bugs (Coccidae) were colonized on potato vines with severe witches' broom, and then transferred to four healthy Bliss Triumph potato plants and two healthy Earliana tomato plants. No evidence of disease transmission was seen.

A common, rather omnivorous species of greenhouse aphid was colonized for 24 days on potato vines which showed secondary symptoms of witches' broom. Many of the aphids were then allowed to feed for 34 days on the sprouts of two Irish Cobbler potato tubers which were laid under the vines of the diseased plant. The tubers were then planted, and progeny from one of the resulting plants was grown later in the greenhouse. These Irish Cobbler plants developed no symptoms of disease. Nine other attempts to transmit the disease with aphids failed.

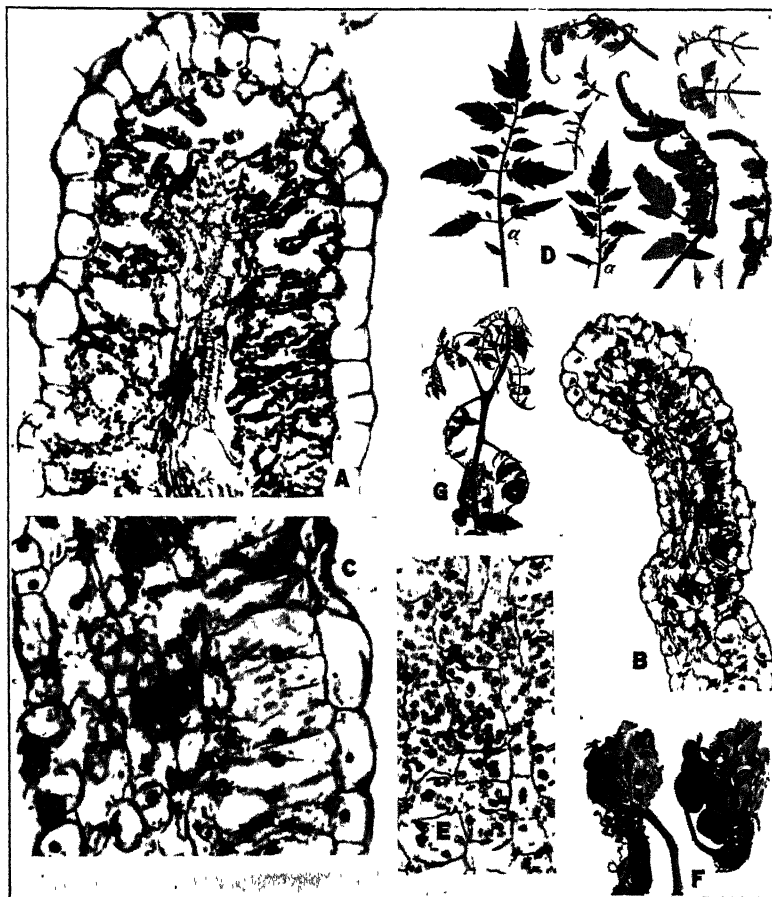
In the field in 1925 and 1926, normal Bliss Triumph potato plants in the rows grew beside others severely affected with witches' broom. Since aphids were abundant on both the healthy and diseased plants late in the season, tubers from eight of the normal plants were planted in the greenhouse at different times. All the resulting plants remained healthy. These experiments in the field and in the greenhouse were too limited to prove that transmission by aphids does not occur. Dana (5) reported some indications that green aphids transmit the disease.

The leaves and stems of potato witches'-broom plants were ground in a sterile food grinder. This inoculum was smeared on the freshly cut surfaces of 4 Russet Burbank and Irish Cobbler seed pieces. The remaining inoculum was then diluted with 100 parts of distilled water, and 8 freshly cut seed pieces of Bliss Triumph, Rural New Yorker, Irish Cobbler, and Russet Burbank potatoes were soaked in the liquid for 30 minutes to 2 hours. The potato vines grown from these 12 seed pieces remained healthy, so these methods of inoculation appear to be ineffective.

## TOMATO WITCHES' BROOM

### SYMPTOMS OF TOMATO WITCHES' BROOM

The symptoms of this disease were seen originally in some tomato stems in arch grafted on potato stems having witches' broom. The first symptom was marginal flavescence in the new leaflets, which is a char-



- A.—Photomicrograph of a cross section of a normal Bonny Best tomato leaf showing the numerous chloroplasts and long palisade cells.  $\times 195$
- B.—Photomicrograph of a cross section of the flavescient margin of a Bonny Best tomato leaf with witches' broom transmitted from a Green Mountain potato. It shows abnormally short palisade cells and relatively few chloroplasts in the chlorenchyma cells.  $\times 176$
- C.—Photomicrograph of a cross section of a completely flavescient leaf of a Bonny Best tomato plant showing the scarcity of chloroplasts, and the dwarfed palisade cells.  $\times 312$
- D.—The two leaves marked *a* were cut from a normal Earliana tomato plant. The other leaves were cut from an Earliana tomato plant with witches' broom transmitted to it from a Perfect Peachblow potato. The diseased leaves show extreme flavescence, curling, dwarfing, and in three figures, absence of leaf blades.  $\times \frac{1}{8}$
- E.—Photomicrograph of a longitudinal section of the stem of a Jersey Peachblow potato with secondary symptoms of witches' broom. It shows minute subspherical, nonstratate starch grains in the pith.  $\times 162$
- F.—Jersey Peachblow potato plant with only flavescient, simple leaves 1 to 4 mm. wide, and stems no longer than 7 cm. at the age of 5 months. These are extreme symptoms of witches' broom.  $\times 1$
- G.—Earliana tomato showing the symptoms of witches' broom transmitted to it through an inarch graft with a Russet Burbank potato having the disease. The leaves are curled, dwarfed, and flavescient.  $\times \frac{1}{8}$



acteristic symptom of witches' broom. The terminal leaflets usually turned light yellow, pale green, hyaline, or purple. The leaflets were much dwarfed and often rugose. Many of them had very narrow leaf blades, or none at all. (Figs. 10, 11, and pl. 1, D, G.) The leaflets and rachises were prominently downward curled, but none were upward rolled. In the first series of grafts the Earliana toma-



FIG. 10.—Flavescent Earliana tomato plant, showing witches' broom transmitted to it by inarch grafting on the stem of a diseased Perfect Peachblow potato. Note the bladeless leaflets and dead ends of compound leaves. This plant grew from a small cutting taken from a witches'-broom tomato plant.  $\times 1/7$

atoes with witches' broom bloomed profusely and produced many small insipid tomatoes containing relatively few seeds. Only a small percentage of these seeds were of normal size and viability. Tomatoes in the later series bore very few fruits. The flowers were normal in appearance and development, except that some were abnormally light in color. Some of these symptoms have been described by Young (24).

In one tomato 1.5 meters tall the symptoms appeared in some branches near the base of the plant within 72 days after grafting,

and did not appear in the top of the plant until 44 days later. Witches'-broom symptoms in the tops of tomatoes were not caused by the constrictions in the grafted stems due to long, tight wrapping, as the same symptoms appeared early in branches attached below the grafts in some stems. The witches'-broom potato stems in the grafts lived until symptoms appeared in many of the tomatoes; some lived longer. A few of the potato stems were cut off below the grafts and then grew as scions on the tomatoes.

Senescence was not much delayed in witches'-broom tomato plants. The normal, large, old leaves, that developed before the appearance of the disease symptoms, die, leaving only the flavescent, curled, nearly bladeless leaves on the stems. (Fig. 10.) The



FIG. 11.—A, top of a normal Earliana tomato plant; B, flavescent top of an Earliana tomato plant showing severe symptoms of witches' broom transmitted to it by inarch grafting with a diseased Russet Burbank potato.  $\times \frac{1}{4}$

senescent stems turned yellow and became hollow, tough, and partly dry. Normal tomato stems were not hollow.

In this senescent and diseased condition some of the witches'-broom tomatoes lived from one to two months before dying. Slender, flavescent, dwarfed branches grew from the axils of many old leaves. The ends of most of the chlorotic, compound leaves died, leaving only the basal leaflets alive.

To test the persistence of the disease, many cuttings from old witches'-broom tomatoes were planted in sand. Most of them died without growing much, which indicated that they originally contained too little stored food. Normal tomatoes were easily propagated by cuttings. A few of the diseased cuttings grew well and continued to exhibit characteristic symptoms of witches' broom. The one shown in Figure 10 grew for 10 weeks.

#### WITCHES' BROOM COMPARED WITH OTHER VIRUS DISEASES OF TOMATO

A comparison of witches' broom with other tomato viroses leads to the conclusion that witches' broom is probably a new disease. Since

the virus came from witches'-broom potatoes, the disease is called tomato witches' broom. It differs conspicuously from tomato yellows which causes upward rolling of the leaves, and does not regularly cause flavescent leaf margins and extremely dwarfed leaflets without leaf blades. Witches' broom is unlike mild mosaic or the mosaic transmitted to tomato from potato rugose mosaic. It does not resemble linear (shoe string) or crinkle mosaic in that the leaves are not mottled or long and slender, the flowers are not abnormal, and the tops do not have a fringed appearance. There is only slight similarity between witches' broom and fernleaf. These other viroses of tomato were described by McKay (13, 14), McKay and Dykstra (16), Shapovalov (21), Weber and Ramsey (22), Johnson (10), and Kraybill and Eckerson (11).

#### EXPERIMENTS IN TRANSMISSION OF WITCHES' BROOM FROM POTATOES TO TOMATOES

The transmission of the witches'-broom virus between potatoes and tomatoes are shown in Table 1. Thirty-eight ungrafted Bonny Best and Earliana control tomatoes, 28 of which were in the pots with the grafted tomatoes, remained free from witches' broom while they were under observation for two to four months. Besides these, all four plants in the two sets of normal Bonny Best tomatoes grafted together did not show witches' broom while they were watched for 107 days. About one-fourth of the grafted and control tomatoes showed traces of very mild mosaic, but the mottling did not obscure the witches'-broom symptoms. These grafts were made in two series in the greenhouse in 1927.

TABLE 1.—*Transmission of witches' broom involving tomatoes*

Variety with witches'-broom disease	Variety grafted upon	Number of inoculations	Number of inoculations causing disease	Length of incubation period
Perfect Peachblow potato.....	Earliana tomato.....	5	3	53 days.
Green Mountain potato.....	do.....	1	1	49 days.
Do.....	Bonny Best tomato.....	11	6	35 to 82 days.
Colorado Pearl potato.....	Earliana tomato.....	1	0	
Bliss Triumph potato.....	do.....	7	3	54 to 72 days.
Rural New Yorker potato.....	do.....	5	1	53 days.
Blue Victor potato.....	do.....	2	1	Do.
Russet Burbank potato.....	do.....	1	1	Do.
Six Weeks potato.....	Bonny Best tomato.....	1	1	35 days.
Earliana tomato.....	Bliss Triumph potato.....	4	1	56 days.
Do.....	Russet Burbank potato.....	1	1	63 days.
Do.....	Bonny Best tomato.....	11	6	35 to 84 days.

#### EXPERIMENTS IN SEED TRANSMISSION OF WITCHES' BROOM

In trying to determine the possibility of transmitting the witches'-broom virus through seeds, Earliana tomato plants were grown from seed produced by a tomato with this disease. Sixteen of these plants were kept under observation for 174 days and 19 others for 82 days. Four became abnormal within 27 days and 11 became abnormal later; 6 remained abnormal. The abnormalities consisted of yellowed, rugose lower leaves with necrotic spots, unusually long, twisted teeth on the leaflets, and marginal flavescence. These plants developed

none of the severe symptoms of tomato witches' broom, so these probably were not symptoms of this disease. Although nematodes on tomato roots may cause a chlorosis of the top leaves, none were present on the roots of these plants. The condition caused by nematodes is readily distinguishable from that caused by witches' broom in tomatoes.

In a later series of experiments, 45 Earliana tomato plants were grown from the seed of 6 tomato witches'-broom plants. They showed no symptoms of witches' broom within five months. This fact leads to the conclusion that tomato witches' broom is not transmitted through seeds.

## HISTOLOGICAL SYMPTOMS OF WITCHES' BROOM

### POTATOES

Mitosis was studied in the buds of four varieties of potatoes showing severe symptoms of witches' broom, and in normal potatoes. The nuclei in the resting and mitotic stages appeared to be the same in the normal and in the witches'-broom potatoes. No abnormal cytological structures were seen.

Iodine was used in an attempt to discover a quick method of identifying viroses. It was mixed with drops of juice expressed from stems, and also painted on stems from which some of the cortical tissues had been removed. Abundant starch was shown by quick blackening. While the stems of witches'-broom potatoes usually contained more starch than normal stems, some normal stems contained abundant stored starch, and some stems of witches'-broom potatoes contained very little starch, so iodine was not very reliable in diagnosis.

Much of the starch seen in the stems was different from tuber starch in that the stem-starch grains were nearly spherical and showed a few lines radiating from the clear centers, rather than being sub-elliptical with concentric, eccentric striae like tuber starch. (Pl. 1, E.) Grains resembling those of tuber starch were sometimes seen in potato stems.

### TOMATOES

Sections of normal and witches'-broom tomato leaves exhibited clear differences between normal and flavescent regions. Normal tomato leaves were 200-325 microns in diameter, with palisade cells 40-70 microns long. The chlorenchyma cells contained numerous chloroplasts. (Pl. 1, A.) In sharp contrast to the normal leaves were the entirely flavescent leaves of diseased plants. These were only 95-200 microns thick, with palisade cells only 25-40 microns long. The region of spongy parenchyma was thin. Very few chloroplasts were seen, which clearly explains the hyaline yellow color of the leaves. (Pl. 1, C.)

Sections of leaves showing marginal flavescence revealed distinct morphological modifications. (Pl. 1, B.) The leaves were only 70-150 microns thick. The palisade cells of the interior of the leaf were 40-50 microns long, but at the margin of the leaves they were only 17-30 microns long. Furthermore, most of these marginal palisade cells were circular to broadly triangular in outline. Abnormally few chloroplasts were present, particularly in the marginal cells, which explains the marginal flavescent color. Intercellular spaces were



abnormally numerous in the flavescent leaves. In the normal leaves the palisade cells at the margin were the same length as those in the interior of the leaves. These sections of tomato leaves showed a clear morphological basis for marginal flavescence. Perhaps because only old material was available, sections of normal and witches'-broom potato leaves did not show such differences.

#### DISCUSSION

Witches' broom is one of the unmottled viroses that severely injure affected plants. Potatoes attacked by it produce few or no marketable tubers when the tops show prominent symptoms. Tomatoes attacked by it produce no fruit, or poorly flavored small fruits of no value.

Three symptoms are common in witches' broom of potato and tomato: Extreme leaf dwarfing, marginal flavescence of the leaves, and abnormally numerous axillary branches. The disease in these hosts is known to have the same cause, for the virus was directly transmitted from witches'-broom potatoes to tomatoes, and then returned to potatoes in which it produced characteristic symptoms.

Controlled transmissions have been accomplished only by keeping diseased and healthy tissues in close contact for a few weeks. All attempts to transmit the virus by leaf-mutilation inoculations and through the freshly cut surfaces of seed pieces failed. This virus seems to be like the virus of peach yellows in that it is difficult to transmit.

The witches'-broom virus selected in eight hills of potatoes in 1925 was transmitted many times. In one case the virus from a Bliss Triumph potato was transmitted by a tuber plug to a Russet Burbank potato, from which it was transmitted later by an inarch graft to an Earliana tomato. By a stem graft the virus was returned to Bliss Triumph and Russet Burbank potatoes. This exemplifies the way in which the successive transfers of the virus occurred.

Symptoms due to other causes sometimes suggest potato witches' broom. The abnormally numerous axillary branches sometimes borne by potatoes with severe spindle tuber are normally green and not spindling; so they are unlike the axillary branches of witches'-broom potatoes.

Spindling sprout is a characteristic symptom of witches' broom. Tubers from leaf-roll potatoes sometimes produce this symptom too, but the subsequent development of the sprouts is very different in witches' broom. Physiological disturbances sometimes cause spindling sprouts to appear. Whipple (23) secured some evidence of this phenomenon. It was observed by the present authors that rotting of seed pieces often caused the resulting sprouts to be very spindling. Hence spindling sprouts seem to be a symptom rather than a separate disease.

The aerial tubers caused by *Corticium vagum* usually are larger than those caused by witches' broom. This is the only important symptom common to both diseases.

The primary symptoms of leaf roll in potato tops may resemble witches' broom for only a short time. Combinations of the two diseases are recognizable.

Richards, Blood, and Linford<sup>9</sup> described a new disease in Utah which has some symptoms that closely resemble those of potato witches' broom. In private conversation, Richards described symptoms of the new disease which clearly distinguish it from witches' broom.

Kunkel (12) states that aster yellows is distinct from potato witches' broom.

#### SUMMARY

Potato witches' broom was discovered in Montana in 1915.

It is so destructive that the percentage of loss is usually the same as the percentage of visible infestation.

The primary symptoms of potato witches' broom are: Dwarfed, flavescent top leaves, often with yellow margins; upper parts of stems cylindrical, with swollen nodes; rapid elongation of stems; profuse branching of the tops; aerial tubers; basal sprouts from tubers; and numerous small subterranean tubers. These symptoms intergrade with the secondary symptoms.

The main secondary symptoms are: Flavescence and purpling of the tops; marginal flavescence and dwarfing of the leaflets; simple leaves; numerous, spindling axillary branches; slender, cylindrical stems with enlarged nodes; filamentous stems; spindling sprouts from tubers or the base of the stem; aerial tubers; and numerous, very small subterranean tubers.

The disease is tuber perpetuated, but the exact means of dissemination in the field is unknown.

Tubers from witches'-broom potatoes appear to have no period of dormancy.

Senescence is delayed in potato vines affected with witches' broom.

In 42 per cent of the 142 trials made, potato witches' broom was transmitted by tuber-core grafts to 11 varieties of potato. It was transmitted twice by stem grafts.

Tuber core grafts with normal cores and seed pieces did not cause abnormalities in the resulting plants.

Seventy-one leaf-mutilation inoculations all failed to transmit potato witches' broom.

The disease was not transmitted by aphids or mealy bugs, nor was it transmitted by soil, or by root or leaf contact.

The appearance of the symptoms of viroses was often hastened by cutting off the ends of the stems of plants before signs of senescence appeared.

Witches' broom was transmitted by inarch grafts from potatoes to 17 tomatoes, from tomatoes to five other tomatoes, and from tomatoes back to two potatoes.

The main symptoms of tomato witches' broom are: Flavescence of the tops, marginal flavescence of the leaflets, extreme dwarfing, curling, flavescence and purpling of the leaflets, partial or total absence of leaf blades, spindling, flavescent axillary branches, and insipid little tomatoes.

Tomato witches' broom appears to be a new disease.

<sup>9</sup> LINFORD, M. B. FURTHER OBSERVATIONS ON UNKNOWN POTATO DISEASE IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11: 110-111. 1927. [Mimeographed.]

RICHARDS, E. L., BLOOD, H. L., and LINFORD, M. B. DESTRUCTIVE OUTBREAK OF UNKNOWN POTATO DISEASE IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11: 93-94. 1927. [Mimeographed.]

Sections of tomato leaves showed a prominent morphological basis for marginal flavesence. The palisade cells were abnormally short, little chlorophyll was present, and the margins of the leaves were abnormally thin.

## LITERATURE CITED

- (1) APPEL, [O.]  
1926. GELBFLECKIGKEIT DER KARTOFFELSTAUDEN. Deut. Landw. Presse 53: 209, illus.
- (2) BISBY, G. R., and TOLAAS, A. G.  
1920. POTATO DISEASES IN MINNESOTA. Minn. Agr. Expt. Sta. Bul. 190, 44 p., illus.
- (3) COONS, G. H., and KOTILA, J. E.  
1923. MICHIGAN POTATO DISEASES. Mich. Agr. Expt. Sta. Spec. Bul. 125, 55 p., illus.
- (4) CUTLER, G. H., and SANFORD, G. B.  
1921. POTATO DISEASES. Alberta Univ., Col. Agr. Field Husb. Circ. 7, 23 p., illus.
- (5) DANA, B. F.  
1926. MOSAIC AND RELATED DISEASES OF POTATO AND OTHER CROPS. Wash. Agr. Expt. Sta. Bul. 208: 33-34.
- (6) GOSS, R. W.  
1926. A SIMPLE METHOD OF INOCULATING POTATOES WITH THE SPINDLE-TUBER DISEASE. Phytopathology 16: 233.
- (7) GÜSSOW, H. T.  
1918. OBSERVATIONS ON OBSCURE POTATO TROUBLES. IV. MOSAIC DISEASE TRANSFERRED BY INARCHING. Phytopathology 8: 495.
- (8) HUNGERFORD, C. W., and DANA, B. F.  
1924. WITCHES' BROOM OF POTATOES IN THE NORTHWEST. Phytopathology 14: [372]-383, illus.
- (9) JACZEWSKI, A. A.  
1926. [WITCHES' BROOM OF THE POTATO.] Materials Mycol. and Phytopath. 5 (2): 117-128, illus. [In Russian. Abstract in Rev. Appl. Mycol. 6: 746, 1927.]
- (10) JOHNSON, J.  
1927. THE CLASSIFICATION OF PLANT VIRUSES. Wis. Agr. Expt. Sta. Research Bul. 76, 16 p., illus.
- (11) KRAYBILL, H. R., and ECKERSON, S. H.  
1927. TOMATO MOSAIC. FILTRATION AND INOCULATION EXPERIMENTS. Amer. Jour. Bot. 14: 487-495, illus.
- (12) KUNKEL, L. O.  
1928. FURTHER STUDIES ON THE HOST RANGE OF ASTER YELLOWS. (Abstract) Phytopathology 18: 156.
- (13) MCKAY, M. B.  
1921. MOSAIC DISEASE OF TOMATOES. Oreg. Agr. Expt. Sta. Crop Pest and Hort. Rpt. (1915/20) 3: [179]-184, illus.
- (14) ———  
1921. WESTERN YELLOW TOMATO BLIGHT. Oreg. Agr. Expt. Sta. Crop Pest and Hort. Rpt. (1915/20) 3: [174]-178, illus.
- (15) ———  
1922. POTATO DISEASES IN OREGON AND THEIR CONTROL. Oreg. Agr. Expt. Sta. Circ. 24, 53 p., illus.
- (16) ——— and DYKSTRA, T. P.  
1927. SUGAR BEET CURLY-TOP VIRUS, THE CAUSE OF WESTERN YELLOW TOMATO BLIGHT. (Abstract) Phytopathology 17: 39.
- (17) McLARTY, H. R.  
1926. WITCHES' BROOM OF POTATOES. Sci. Agr. 6: 394-395, illus.
- (18) MURPHY, P. A., and M'KAY, R.  
1926. METHODS FOR INVESTIGATING THE VIRUS DISEASES OF THE POTATO, AND SOME RESULTS OBTAINED BY THEIR USE. Roy. Dublin Soc. Sci. Proc. (n. s.) 18: 169-184, illus.
- (19) SANFORD, G. B.  
1924. POTATO DISEASES. Alberta Univ., Col. Agr. Bul. 5, 34 p., illus.
- (20) SCHULTZ, E. S., and FOLSOM, D.  
1923. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES. Jour. Agr. Research 25: 43-118, illus.

- 
- (21) SHAPOVALOV, M.  
1927. INOCULATION EXPERIMENTS WITH WESTERN YELLOW TOMATO BLIGHT IN RELATION TO ENVIRONMENTAL CONDITIONS. (Abstract) *Phytopathology* 17: 746.
- (22) WEBER, G. F., and RAMSEY, G. B.  
1926. TOMATO DISEASES IN FLORIDA. *Fla. Agr. Expt. Sta. Bul.* 185, p. [61]-138, illus.
- (23) WHIPPLE, O. B.  
1919. DEGENERATION IN POTATOES. *Mont. Agr. Expt. Sta. Bul.* 130, 29 p., illus.
- (24) YOUNG, P. A.  
1927. TRANSMISSION OF POTATO WITCHES' BROOM TO TOMATOES AND POTATOES. (PRELIMINARY REPORT). *Science* (n. s.) 66: 304-306.

# THE COMPARATIVE NUTRITIVE VALUE OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL FOR DIFFERENT ANIMALS<sup>1</sup>

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## INTRODUCTION

There is need for information which will make possible the application of the knowledge of the qualitative differences in proteins to the selection of economical rations for farm animals. It is generally understood that animal protein is of higher quality than vegetable, and that a combination of the two is more efficient than vegetable protein alone. This knowledge is commonly made use of in pork and egg production. Within recent years, however, more information has been accumulating on the use of vegetable proteins, which tends to show that protein concentrates of vegetable origin can be frequently used to advantage to the partial or entire exclusion of the animal protein fraction. In this respect the two vegetable protein feeding stuffs, linseed meal and cottonseed meal, have held a great deal of interest and are extensively used as feeds for livestock.

In many feeding trials, especially those conducted in the North, linseed meal has been used as a standard protein feed, since it was felt that the protein of cottonseed meal was poorer in quality and that the cottonseed meal was of a toxic character. There is, therefore, no entire agreement as to the respective protein value of these two meals. In the majority of the experiments these vegetable protein feeds were investigated without taking into consideration feed consumption. In many instances the respective meals were taken from different shipments or sources and unknown factors were thus introduced, such as variable toxicity and nutritive value, which detracted from the clarity of the results. Hence, a further study of the comparative nutritive value of the proteins of cottonseed and linseed meal was considered necessary; such a study constitutes the subject of the present report.

## REVIEW OF LITERATURE

The digestibility of the proteins of cottonseed meal is reported by Fraps (4)<sup>2</sup> to be 88.4 per cent in the case of sheep and steers, and by Lindsey and his coworkers (8) as 82.19 to 84 per cent for sheep. Mendel and Fine (14), who employed dogs as experimental animals, state that the protein of cottonseed flour is digested to the extent of 67 to 75 per cent, compared to 91 per cent for the proteins of meat. Rather (23), using men as subjects, found an average protein digestibility of 77.6 per cent for cottonseed meal and 78.4 per cent for cottonseed flour, in contrast to 96.6 per cent for the protein of meat.

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 870.

In comparing the nutritive value of the proteins in cottonseed meal, alfalfa hay, and corn, Nevens (17) found that when one of these feeds furnished the sole source of protein in an otherwise complete ration, to the extent of 10 per cent, the utilization of the proteins for the growth of albino rats was 66, 62, and 49 per cent, respectively. That cottonseed meal and flour were satisfactory sources of protein for the growth of albino rats, when these feeds furnished 18 per cent or more protein in the ration, was found by Richardson and Green (24, 25, 26). Osborne and Mendel (19) also reported that normal growth was procured for a considerable period in rats when 18 per cent of cottonseed globulin was fed in the ration. These latter investigators (18) found cottonseed flour of value in supplementing the proteins of corn gluten for growth of chickens. In other studies on the value of certain proteins and protein concentrates as supplements to corn gluten, these authors (20) demonstrated that the proteins extracted from cottonseed flour by a sodium hydroxide solution were efficient supplements to the proteins of corn gluten for the growth of rats. McCollum and Simmonds (11) reported that rats maintain their body weight when fed a ration containing 6 per cent of protein derived from cottonseed.

In studying the relation of the quality of proteins to milk production, Hart and Humphrey (5) found that the proteins of gluten feed, linseed meal, distillers' grains, and cottonseed meal were of equal value in supplementing the proteins of corn meal and alfalfa hay. Later in similar experiments (6) the proteins of distillers' grains, linseed meal, and gluten feed proved more efficient than cottonseed meal protein. The proteins of the feeding stuff tested formed but 40 per cent or less of the protein content of the ration, and the results were calculated upon the basis of total nitrogen absorbed by the animals.

Maynard, Fronda, and Chen (13), in studying the protein efficiency of combinations of corn meal and certain vegetable protein feeding stuffs, found that a mixture of corn meal and cottonseed meal was as efficiently utilized for growth of rats as a similar protein combination of corn meal and linseed meal.

That cottonseed and cottonseed meal may prove injurious to different species of animals has been shown by numerous investigators. The first assumption that the high protein content of cottonseed meal is responsible for its harmful effects was disproved by Dinwiddie (3), who showed that this theory was not supported by a study of the recorded feeding trials. Withers and Brewster (30) first attributed the toxic principle of cottonseed meal to a certain group of the protein molecule which contained loosely bound sulphur. Later work by Withers and Carruth (31, 33) led them to conclude that the toxicity was due to the presence of "gossypol." They were also of the opinion that gossypol may be changed to a nearly related substance, "D-gossypol," which possesses somewhat different chemical properties. The reduction of the toxicity of cottonseed meal by heat was believed by these investigators to be due to a change in the gossypol protein compound which renders it nontoxic or impossible for the animal to digest. These workers (32) also observed that iron salts have an antidotal action on cottonseed-meal poisoning in swine and rabbits. The theory that cottonseed meal injury is due to the pres-

ence of gossypol is strengthened by the work of Schwartze and Alsberg (27).

In a series of feeding experiments with rats, Osborne and Mendel (19) and Richardson and Green (24, 25, 26) observed no toxic effects with cottonseed meal or flour, but the cottonseed kernels proved injurious. Nevens (17) likewise found no toxic effects from feeding cottonseed meal to rats.

The feeding value of linseed meal for livestock has been demonstrated on numerous occasions. That this feed carries proteins which are of supplemental value to carbonaceous feeds is generally accepted by investigators and feeders. Henry and Morrison (7, p. 723) report an average digestibility of 89 per cent for the proteins of linseed meal. McCollum, Simmonds, and Parsons (12) observed that a ration of 25 per cent linseed meal, 1 NaCl, 1.3 CaHPO<sub>4</sub>, 2 butterfat, and dextrin to 100 produced fair growth in rats. The growth and well-being of the rats was not comparable, however, to that on a ration containing 6 per cent protein from rye and 3 per cent from linseed meal. The latter ration also proved superior to 9 per cent rye protein. The combination of rye and linseed meal gave greater average growth than 8 per cent milk protein. These rations, in the light of present knowledge, were low in vitamins A and B. Nevertheless, they show the possible supplementing value of linseed meal. Further work by McCollum and Simmonds (11) shows that 8 per cent of flaxseed protein just serves to maintain body weight in the rat. This ration also was deficient in vitamin B.

That linseed meal may prove toxic to certain species of animals was reported by Almy and Robinson (1). These investigators found that ingested linseed meal brings about a nervous and excitable state in brook trout, causes them to become black and blind, and proves fatal to a large percentage of fish. They ascribed the injurious effect in large measure to hydrocyanic acid which is present in most linseed meals as the result of the hydrolysis of a cyanogenetic glucoside, phaseolunatin. The reason that most linseed meals apparently prove nontoxic is because decomposition of the glucoside does not take place in the stomach on account of the destruction of the enzyme by the high temperatures used in the expression of the oil.

#### EXPERIMENTAL METHODS

A large number of studies have been made with rats to ascertain the relative quality and adequacy of the proteins furnished by various sources. However, there is by no means entire agreement as to what constitutes a satisfactory method for determining the relative or comparative values of different protein sources, and many of the results reported by different investigators are conflicting. It was planned to attack the problem in several different ways to overcome the difficulties that might arise with each individual method, especially since the sources of protein did not differ greatly in quality, as they evidently do in many of the feeding stuffs of vegetable origin.

Protein sources have been compared, by many investigators, on the basis of their relative ability to cause growth or growth and reproduction without taking into consideration possible variations in food consumption. Osborne and Mendel (21) have shown that

any conclusions based on growth curves alone may be wholly misleading. McCollum (9), on the other hand, has pointed out that a rat eats according to its calorific requirements, and consequently food intakes should be comparable where rations are similar in calorific value. Under such conditions individual food intake records would add little of value. Other work has convinced the writers that other factors besides calorific requirements influence food intake, especially where rations differ markedly in physical character and ingredients. Questions of toxicity, palatability, and suitability of the ration to the animal arise. Under such conditions it is necessary to know whether an individual is actually eating enough food for growth before a given ration can be listed as unsatisfactory as to protein.

It was planned to pattern the first studies after those of Osborne and Mendel (22), feeding the rations *ad libitum* and then comparing results on the basis of gain per gram of protein eaten. Such a plan involves the feeding of rations adequate for normal growth with respect to all factors except protein. Also all results used for comparison must come from planes of protein intake producing fairly rapid growth, yet not average normal growth, since gain per gram is an accurate measure only in rations causing fairly good growth because it approaches zero as growth approaches zero. If, on the contrary, growth was comparable to normal there would be no way of ascertaining whether the protein intake was larger than necessary. Any excess protein would lower the true gain per gram. On this basis a plane of protein of approximately 10 per cent was decided upon. At this level rats of the writers' breeding will grow at about two-thirds to three-quarters of the normal rate when linseed meal and cottonseed meal are used as the sole source of protein.

The rats used were from two litters of six each which had been reared in the laboratory under standardized conditions. These were taken at the age of 25 days, weighing from 56 to 66 gm., and were equally distributed as to litter and sex. Each rat was confined in an individual cage and received the ration *ad libitum*.

#### EXPERIMENTS WITH RATS

Three rats from each litter were fed a ration of linseed meal 30 parts, cornstarch 59, salt mixture 4, Crisco 5, and cod-liver oil 2. The three remaining rats in each of the two litters received a similar ration in which the linseed meal was replaced by such a quantity of cottonseed meal as to make the total protein intake comparable. It was constituted as follows: Cottonseed meal 24.2, cornstarch 64.8, salt mixture 4, Crisco 5, and cod-liver oil 2. Vitamin B was supplied by 0.50 gm. of a brewer's yeast fed separately to each animal daily. The salt mixture employed was that described by McCollum (10, *p.* 191) with the addition of 0.25 per cent potassium iodide. Accurate accounts were maintained of the individual weekly food intakes.

The rats were continued on test for 15 weeks, because it seemed desirable that the experimental period should be as long as possible without extending into the period when the rate of growth starts to decrease. The results are shown in Table 1. The proteins of linseed and cottonseed meal appear to be equally efficient for growth of the rat when fed on a 10 per cent protein basis. No difference in the



behavior or appearance of the rats was observed. It is of interest to note that female rats utilized the protein of the rations less efficiently than the males. Although the yeast fed daily furnished some protein which supplemented those of linseed and cottonseed meal, the results, nevertheless, should be comparable, since the same quantity (0.50 gm.) was supplied each individual.

TABLE 1.—*Comparative efficiency for growth of rats of the protein in linseed meal and cottonseed meal*

Ration	Rat No.	Sex	Food intake	Protein intake	Gain in 15 weeks	Gain per gram of protein
Linseed meal, 30 parts; starch, 59; Crisco, 5; salt mixture, 4; cod-liver oil, 2; 0.5 gm. yeast daily.	3036	Female	Gm. 1,043	Gm. 106.5	Gm. 136.0	Gm. 1.27
	3037	Male	1,111	113.5	180.0	1.58
	3038	Female	1,139	116.4	136.0	1.17
	3039	Male	1,091	111.5	171.0	1.53
	3040	Female	968	98.9	104.0	1.05
	3041	Male	1,307	133.5	179.0	1.34
	Average		1,110	113.4	151.0	1.32
Cottonseed meal, 24.2 parts; starch, 64.8; Crisco, 5; salt mixture, 4; cod-liver oil, 2; 0.5 gm. yeast daily.	3042	Female	1,169	119.1	121.0	1.01
	3043	Male	1,187	121.0	193.0	1.59
	3044	Female	1,146	116.8	140.0	1.20
	3045	Male	1,148	117.0	156.0	1.33
	3046	Female	1,219	124.2	133.0	1.07
	3047	Male	1,182	120.5	162.0	1.34
	Average		1,175	119.7	150.9	1.26

Before the foregoing study was completed the writers thought it advisable to obtain data on the digestibility of these two feed stuffs, having in mind that any variation in nutritive value might be partly or wholly due to the digestibility of the feed. Incidentally, the biological value of the feed stuffs was determined according to the method described by Mitchell (15). While the writers are aware of the shortcomings of any method which attempts to assign absolute figures to digestibility or biological values, they are, nevertheless, of the opinion that results secured in this way should prove of value in connection with other biological studies.

For these trials, one litter of 6 rats (3 males and 3 females) which had been reared under standardized laboratory procedure and weighed from 100 to 130 gm. were used. Each animal was confined in a 7-inch cylindrical wire metabolism cage provided with a false screen bottom, set in a crystallizing dish. The urine was absorbed by filter papers placed in the bottom of the dish. The feces were collected on a fine wire screen which was placed from one-half to three-quarters of an inch below the screen bottom. The urine and feces were collected daily and properly preserved to prevent loss of nitrogen. Food scattered by the animals was promptly removed from the filter paper and taken into account. The rations fed were of the same composition as those used in the first series, except that the yeast was fed mixed in the ration—6 per cent yeast replacing an equivalent quantity of starch. A total of two periods of seven days each was obtained with each animal on the two experimental rations. Periods 2 and 3 and 4 and 5 were run successively on the same ration. At the close of the third period, in each case, the order of the ration was reversed, and again two weeks' collection obtained. For example,

rat No. 1, which received the linseed-meal ration the second and third periods, was placed on the cottonseed-meal ration at the close of the third period. Likewise rat No. 4, which received the cottonseed-meal ration the second and third periods, was changed to the linseed-meal ration at the close of the third period. The low nitrogen periods at the beginning and end of the experiment were obtained with a synthetic ration containing the same amount of yeast (6 per cent) as was used in the experimental rations. A preliminary period of seven days preceded the collection periods whenever the rations were changed. The data are recorded in Table 2. The calculations were made according to the method of Mitchell, Beadles, and Keith (16), which takes into consideration the weight and food consumption of the animal in computing its metabolic and endogenous nitrogen. Considerable variation in digestibility and biological values occurred among the individual rats on the two rations. No significant difference was observed between the two meals when the average of the 12 trials in each case are considered. Apparently cottonseed meal and linseed meal, as used in this experiment, are equal in biological value and digestibility for rats.

TABLE 2.—*Nitrogen metabolism data for rats fed rations containing different percentages of nitrogen, together with data showing digestibility and biological value of linseed meal and cottonseed meal*

PERIOD 1									
Ration	Rat No.	Initial weight	Final weight	Food intake	Food N	Fecal N	Urinary N	Digestibility*	Biological value
Low nitrogen ration, containing 0.58 per cent N.		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		
	1	130	134	90.0	-----	0.160	0.275	-----	-----
	2	100	104	72.0	-----	.144	.285	-----	-----
	3	126	132	84.0	-----	.152	.291	-----	-----
	4	105	119	85.0	-----	.171	.264	-----	-----
	5	124	131	100.0	-----	.192	.241	-----	-----
	6	101	105	78.0	-----	.137	.292	-----	-----
PERIOD 2									
Linseed meal, containing 2.17 per cent N.	1	152	173	101.5	2.203	0.494	0.877	85.79	71.04
	2	111	124	84.5	1.400	.365	.611	83.07	75.92
	3	140	157	85.0	1.845	.349	.685	89.43	79.03
PERIOD 3									
Linseed meal, containing 2.17 per cent N.	1	173	189	109.0	2.365	0.503	0.933	86.93	73.30
	2	124	131	75.0	1.628	.352	.784	87.47	70.44
	3	157	178	101.0	2.191	.509	.839	85.12	75.66
PERIOD 4									
Linseed meal, containing 2.17 per cent N.	4	160	182	76.0	1.649	0.420	0.947	83.38	58.62
	5	212	220	92.0	1.996	.503	.985	84.27	65.46
	6	156	164	75.0	1.628	.362	.831	85.81	72.73

\* Corrected for metabolic nitrogen.

TABLE 2.—*Nitrogen metabolism data for rats fed rations containing different percentages of nitrogen, together with data showing digestibility and biological value of linseed meal and cottonseed meal*—Continued

PERIOD 5									
Ration	Rat No.	Initial weight	Final weight	Food intake	Food N	Fecal N	Urinary N	Digestibility	Biological value
Linseed meal, containing 2.17 per cent N.	4	Gm. 162	Gm. 172	Gm. 82.0	Gm. 1.779	Gm. 0.484	Gm. 0.904	81.51	64.62
	5	220	214	78.0	1.693	.356	.945	88.66	64.09
	6	164	170	75.0	1.628	.347	.773	86.73	78.47
Average								85.68	70.83
PERIOD 2									
Cottonseed meal, containing 2.26 per cent N.	4	131	137	86.0	1.944	0.472	0.735	84.47	74.48
	5	147	180	121.0	2.735	.602	.839	86.65	77.55
	6	124	133	82.0	1.853	.402	.728	86.08	77.05
PERIOD 3									
Cottonseed meal, containing 2.26 per cent N.	4	137	147	84.0	1.898	0.491	0.803	82.77	70.15
	5	180	204	124.0	2.802	.725	1.075	82.98	69.29
	6	133	144	88.0	1.989	.501	.822	82.55	73.75
PERIOD 4									
Cottonseed meal, containing 2.26 per cent N.	1	214	232	119.0	2.689	0.675	1.066	82.74	73.53
	2	153	158	80.0	1.808	.482	1.128	81.97	53.98
	3	198	211	113.0	2.554	.657	1.049	82.30	72.74
PERIOD 5									
Cottonseed meal, containing 2.26 per cent N.	1	232	254	120.0	2.710	0.679	1.115	82.77	73.70
	2	158	172	85.0	1.921	.451	.900	85.11	74.43
	3	211	247	127.0	2.870	.735	1.110	82.40	75.81
Average								83.57	72.20
PERIOD 6									
Low nitrogen ration, containing 0.58 per cent N.	1	234	235	111.0	-----	0.196	0.511	-----	-----
	2	160	164	99.0	-----	.190	.478	-----	-----
	3	214	210	98.0	-----	.177	.502	-----	-----
	4	161	146	72.0	-----	.134	.359	-----	-----
	5	190	175	44.0	-----	.094	.340	-----	-----
	6	160	160	80.0	-----	.139	.449	-----	-----

Because of the fact that no difference was observed between linseed and cottonseed meal in digestibility, biological value, or in the gain of rats per gram of protein intake, it was planned to study the comparative supplementing value of these feeds when fed in combination with corn. While there is a possibility that these protein concentrates might supplement one protein in one order and another protein in the opposite order, corn was selected as the protein source for which supplementing sources were to be sought because it is one of the most extensively and commonly used feeding materials, and also because it is generally conceded to contain proteins of poor quality.

The linseed and cottonseed meals were fed in three varying levels with yellow corn in an otherwise complete ration, the meals in each case furnishing 6.8, 8.5, and 10.2 per cent protein. The rations were constituted as follows:

Ingredients	Ration 1125	Ration 1126	Ration 1127	Ration 1128	Ration 1129	Ration 1130
Yellow corn.....	72. 00	67. 00	62. 00	72. 00	67. 00	62. 00
Linseed meal.....	20. 00	25. 00	30. 00			
Cottonseed meal.....				16. 17	20. 20	24. 20
Salt mixture.....	4. 00	4. 00	4. 00	4. 00	4. 00	4. 00
Cod-liver oil.....	2. 00	2. 00	2. 00	2. 00	2. 00	2. 00
Yeast.....	2. 00	2. 00	2. 00	2. 00	2. 00	2. 00
Cornstarch.....				3. 83	4. 80	5. 80

The salt mixture used was the one reported by McCollum (10, p. 191) with 0.25 per cent potassium iodide added. Two per cent of yeast was added to make certain that vitamin B would not be a limiting factor.

Several litters of young stock rats weighing from 47 to 60 gm. were divided into 6 groups of 4 each, 2 males and 2 females, and fed the above rations ad libitum. The animals were confined in standard wire cages provided with false screen bottoms. Accurate group feed-consumption records were maintained as well as individual weekly weights. The results are, in part, shown in Figure 1.

From this figure it is apparent that the rats grew at a nearly normal rate. The growth was fairly comparable on all six rations. Likewise, there was no difference in the behavior or appearance of the various lots. The rats fed cottonseed meal were fully comparable to those fed linseed meal. More cases of reproduction and slightly greater growth occurred in the lots fed cottonseed meal; however, this difference is not regarded as significant.

During the 24 weeks the various groups were on experiment, the 6 females in the three lots fed linseed meal gave birth to 11 litters, or a total of 76 young, of which 85 per cent were weaned at 24 days at an average weight of 25.4 gm. The 6 females on the cottonseed meal-corn combination gave birth to 15 litters or 105 young, of which 73 per cent were successfully weaned at 24 days and these weighed on an average 29.3 gm. It is interesting to note that the reproductive history of these rats does not coincide with the common belief among cattle feeders that cottonseed meal markedly interferes with normal reproduction.

#### EXPERIMENTS WITH RATS AND PIGS

All of the studies thus far had been carried out on one species, the rat, and indicated that with this animal the proteins of linseed meal and cottonseed meal were uniformly well digested, similar in biological value, and equally efficient in supplementing the proteins of corn. The writers desired, however, to obtain information on the comparative response of different species of animals when fed identical rations containing the two feeding stuffs in question. Accordingly,

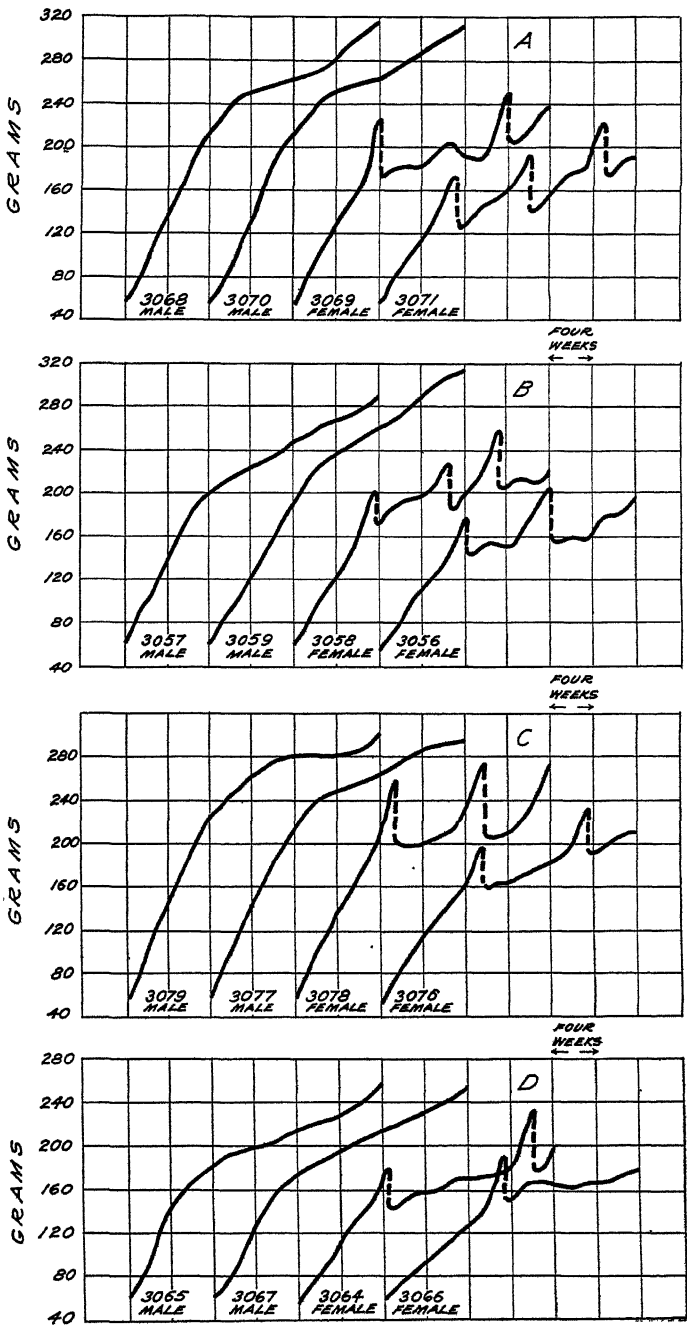


FIG. 1.—Graph showing the value of the protein of corn as a supplement to that of cottonseed meal and linseed meal in growth experiments with rats

A, ration 1128: Yellow corn, 72 parts; cottonseed meal, 16.17; salt mixture, 4; yeast, 2; cornstarch, 3.83; cod-liver oil, 2. B, ration 1125: Yellow corn, 72 parts; linseed meal, 20; salt mixture, 4; yeast, 2; cod-liver oil, 2. C, ration 1130: Yellow corn, 62 parts; cottonseed meal, 24.2; salt mixture, 4; yeast, 2; cornstarch, 5.8; cod-liver oil, 2. D, ration 1127: Yellow corn, 62 parts; linseed meal, 30; salt mixture, 4; yeast, 2; cod-liver oil, 2.

3 lots of 4 each of young stock rats, and 3 lots of 5 each of young weanling pigs weighing on an average 31.7 pounds, were fed identical rations. The pigs were confined indoors in concrete-paved pens and self-fed the respective ration. The rats were kept in the rat room in wire cages provided with false screen bottoms and fed the rations ad libitum. The rats were weighed every week and the pigs every two weeks. Fresh water was supplied daily in both cases.

One lot of rats and pigs received a ration of yellow corn 73 parts, linseed meal 25, and minerals 2.<sup>3</sup> A second lot received 77.8 parts yellow corn, 20.2 cottonseed meal (equivalent in protein content to 25 parts linseed meal), and 2 minerals. A third lot received a ration similar to that fed lot 1 except that the 25 parts of linseed meal was replaced by an equal quantity of cottonseed meal. The corn, linseed meal, and cottonseed meal were taken from the same source of supply as that in the previous rat studies.

The three lots of rats were continued on their respective rations for 24 weeks. The results are recorded in Figure 2. There was no discernible difference between the lot fed linseed meal and the lot fed cottonseed meal. The rate of growth was not comparable to that of rats fed corn-linseed meal or corn-cottonseed meal in rations fortified with cod-liver oil, yeast, and a synthetic salt mixture. Reproduction occurred among the lots on all three rations, with a high mortality of the young. Only in a few instances did the mother successfully nurse her young to 24 days. No difference was observed in this respect between the cottonseed-meal and linseed-meal lots.

The three lots of pigs, on the other hand, responded wholly differently. (Table 3.) From the beginning of the experiment the lot fed linseed meal ate more feed and continued to do better than the lots fed cottonseed meal. One pig in lot 2 (20.2 per cent cottonseed meal) died on the one-hundred and twentieth day. Another one toward the end of the experiment was inclined to go into convulsions when disturbed. In lot 3 (25 per cent cottonseed meal) two pigs died on the sixty-seventh and seventy-eighth days, respectively. On post-mortem examination all three animals showed a marked hemorrhagic condition of the mesentery—a characteristic of cottonseed-meal injury. The remaining animals were killed, after 132 days on experiment, for post-slaughter examination. No deviations from normal were observed at the time. However, the following morning it was noted that all the cottonseed-meal-fed pigs presented a distinctly yellowish coloration of the skin and internal fat. The linseed-meal-fed pigs, on the other hand, were normal.

TABLE 3.—Data showing the relative value of cottonseed meal and linseed meal in rations fed to three lots of pigs of five each kept in dry lot from August 26, 1926, to January 5, 1927 (132 days)

Lot and ration	Average daily feed	Feed required for 100-pound gain	Average initial weight of pigs	Average final weight of pigs	Average daily gain	Number of pigs that died
Lot 1: Yellow corn, 73 parts; linseed meal, 25; minerals, 2.....	Pounds 3.06	Pounds 374.0	Pounds 31.70	Pounds 139.70	Pounds 0.82	0
Lot 2: Yellow corn, 77.8 parts; cottonseed meal, 20.2; minerals, 2.....	2.32	504	32.00	105.80	.46	1
Lot 3: Yellow corn, 73 parts; cottonseed meal, 25; minerals, 2.....	2.26	439	31.50	107.20	.52	2

Special steamed bone meal 40, ground limestone 40, salt 20.

The difference in the response of the rats and pigs fed identical rations is of interest in that it shows that animals of different species may not respond alike to the same feeds. This does not necessarily imply that a difference in protein requirements or utilization exists between the two species. Questions of differences in palatability,

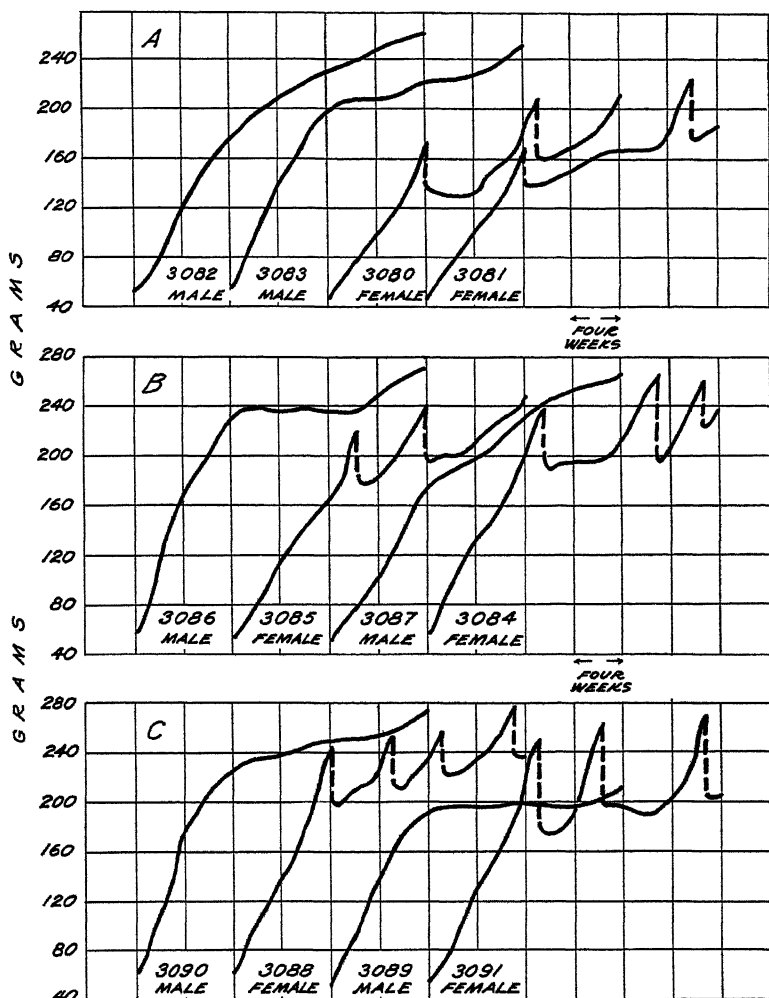


FIG 2.—Graph showing the relative value of the protein of linseed meal and cottonseed meal in growth experiments with rats

- A, ration 1131: Yellow corn, 73 parts; linseed meal, 25; minerals, 2.  
 B, ration 1132: Yellow corn, 77.8 parts; cottonseed meal, 20.2; minerals, 2.  
 C, ration 1133: Yellow corn, 73 parts; cottonseed meal, 25; minerals, 2.

suitability, and toxicity of the ration to the animal arise. The writers are of the opinion that the poor results obtained with cottonseed meal in the case of pigs were primarily due to toxicity and were not complicated by inferior protein. This belief is substantiated by other work at this station which has shown that cottonseed meal

can be profitably fed to pigs when combined with an animal protein feed such as tankage. These latter trials also indicate that the protein in cottonseed meal is equal in biological value to that in linseed meal.

### EXPERIMENTS WITH CHICKS

Both cottonseed meal and linseed meal have been fed to laying hens on a few occasions with conflicting and inconclusive results. No experiments, however, have come to the writers' attention which attempted to compare the proteins of these two feeding materials in the case of the young growing chick. The latter represents a rapidly growing and rather delicate species which responds very readily to poor or good nutrition. Accordingly, it was planned to test the comparative protein efficiency of linseed meal and cottonseed meal when fed to chicks alone and when fed in combination with an animal protein in an otherwise complete ration.

One-week-old White Leghorn chicks of the station stock were divided into four lots of 25 each and placed in 3 by 6 foot experimental pens located indoors. Pine shavings, renewed weekly, were used as litter. The rations were compounded as follows:

	Lot 1	Lot 2	Lot 3	Lot 4
Yellow corn.....	43.5	49.3	50.3	53.2
Wheat middlings.....	20.0	20.0	20.0	20.0
Linseed meal.....	29.5		14.7	
Cottonseed meal.....		23.7		11.8
Meat scraps (50 per cent protein).....			10.0	10.0
Bone ash.....	4.0	4.0	2.0	2.0
CaCO <sub>3</sub> .....	1.0	1.0	1.0	1.0
NaCl.....	1.0	1.0	1.0	1.0
Cod-liver oil.....	1.0	1.0	1.0	1.0

These were fed in the form of a dry, finely ground mash, with water to drink. The rations of lots 1 and 2 represented 10 per cent protein in the form of linseed meal and cottonseed meal, respectively. In the case of lots 3 and 4 one-half or 5 per cent of vegetable protein was replaced by an equal quantity of animal protein. The bone ash in the rations of lots 3 and 4 was reduced to compensate for the bone contained in the meat scraps, since it was desired to maintain approximately the same calcium intake in all four groups.

The results of the chick experiment are summarized in Table 4. At a 10 per cent protein level cottonseed meal proved greatly superior to linseed meal for promoting growth and livability of chicks. Replacing one-half of the two vegetable proteins with an animal protein such as meat scraps (lots 3 and 4) made for a marked improvement in growth and livability. The combination of meat scraps and cottonseed meal proved to be better in this respect than that of meat scraps and linseed meal. Cottonseed meal alone (lot 2), however, was equal to the meat scraps-linseed meal mixture (lot 3). The chicks in lot 1 (10 per cent linseed-meal protein) exhibited a marked intestinal disorder which undoubtedly proved to be a contributing factor to the high mortality and slow growth.



This marked gastrointestinal disturbance was not observed in lot 3, where 5 per cent of the linseed-meal protein was replaced by an equivalent quantity of animal protein. However, the equivalent protein in form of cottonseed meal and meat scraps proved better than the linseed meal-meat scraps combination. Post-mortem examination of the chicks that died revealed nothing of a toxic nature which could be attributed to the meals. On these bases and under the conditions of the experiment it would appear that the protein in cottonseed meal was superior to that in linseed meal for growth of young chicks.

Further work by Kennard <sup>4</sup> at this station indicates that linseed meal may serve as a fairly efficient source of protein for egg production—proving equal to meat scraps in one trial. This fact suggested that probably the age or maturity of the bird was a factor in the difference in response between the chick and hen. To test this hypothesis the writers placed on experiment two lots, 10 each, of 12-week-old White Leghorn pullets. These had been reared indoors on a standard chick ration and were normal in all respects. They were fed rations similar in composition to lots 1 and 2 of the foregoing experiment. Pine shavings, renewed weekly, were used as litter. Water was supplied as a drink.

TABLE 4.—Data for 4 lots of chicks of 25 each kept for 11 weeks on rations containing different quantities of linseed meal or cottonseed meal protein, fed without and with the addition of animal protein

Age of chicks (weeks)	Lot 1, fed 10 per cent linseed-meal protein		Lot 2, fed 10 per cent cottonseed- meal protein		Lot 3, fed 5 per cent linseed-meal protein and 5 per cent meat-scrap protein		Lot 4, fed 5 per cent cottonseed-meal protein and 5 per cent meat-scrap protein	
	Number surviving	Average weight	Number surviving	Average weight	Number surviving	Average weight	Number surviving	Average weight
		Gm.		Gm.		Gm.		Gm.
1.....	25	69.9	25	70.2	25	69.2	25	70.0
3.....	23	97.0	25	121.0	22	115.2	23	148.0
5.....	17	100.7	25	191.5	20	171.7	22	247.7
7.....	10	139.0	* 23	312.5	* 20	291.0	* 22	424.7
9.....	10	167.5	20	449.0	20	422.5	21	567.8
11.....	9	227.2	20	574.5	19	561.0	21	779.9
12.....	8	271.8	20	648.3	18	613.1	21	862.3

\* Cockerel and pullet weights averaged separately after seventh week.

The birds were continued under this management for nine weeks. One bird in each lot died from bronchitis. The gain per bird during the course of the experiment was 311 gm. for those fed linseed meal and 374 gm. for those fed cottonseed meal. Although little significance is attached to the difference of 63 gm. in favor of the birds fed cottonseed meal, it is interesting to note that those fed linseed meal made comparatively small gains the first six weeks. The gains of those receiving cottonseed meal, on the other hand, were gradual. The total feed consumption of the two lots was practically equal. This suggests that maturity of the bird may be a factor in determining the relative nutritive value of these two protein feeds. This fact requires further experimentation.

<sup>4</sup> Unpublished data.

## EXPERIMENTS WITH CALVES

In addition to the data secured with rats, pigs, and chicks, an experiment involving the feeding of these two protein concentrates to calves was carried out. Fourteen beef calves of the Aberdeen-Angus breed, averaging 342 pounds in weight, were fed in 2 lots of 7 each for a period of 280 days. The lots were comparable in weight, age, and sex. Obviously the feed mixtures as a whole could not be identical with those of the rats, pigs, or chicks. Alfalfa hay and corn silage were added. The proteins of these roughages in addition to those of corn were of necessity complicating factors. No concise conclusions as to the biological values of the proteins of cottonseed meal and linseed meal could be drawn when fed in combination with hay, silage, and corn, because the protein intake in the form of the two meals represented but a small portion of the total, and consequently their respective values were masked to a much greater extent than when fed alone or in combination with corn. Nevertheless, the data with calves are presented to show what results might be expected with the same feeds, particularly cottonseed meal, that from all indications had variable effects on different species of animals.

The 2 lots of calves received a ration of alfalfa hay, corn silage, and a grain mixture of 9 parts yellow corn and 1 part of either of the protein feeds. The corn and the cottonseed and linseed meals were from the same shipment as those used in the foregoing experiments with rats, pigs, and chicks. Both the roughages and grain mixture were fed *ad libitum*.

The average daily feed consumption of lot 1 consisted of 6.72 pounds ground corn, 0.71 pound linseed meal, 4.12 pounds alfalfa hay, and 4.86 pounds corn silage, possessing a nutritive ratio of 1:6.6. The individuals in lot 2 consumed on a daily average 6.04 pounds ground corn, 0.64 pound cottonseed meal, 3.71 pounds alfalfa hay, and 4.41 pounds corn silage having a nutritive ratio of 1:6.3.

On these rations, fed for 280 days, the calves of lot 1 (linseed meal) made an average daily gain of 1.70 pounds, and those of lot 2 (cottonseed meal) 1.66 pounds. The feed required per unit gain was slightly less for those fed cottonseed meal (lot 2). No unfavorable effects that might be traced to the rations were noticed in either lot. In numerous experiments with fattening beef cattle at the Wisconsin,<sup>5</sup> Pennsylvania (29), Nebraska (28), Kansas,<sup>5</sup> and Iowa (2) stations, linseed meal, with few exceptions, has produced greater and more economical gains than has cottonseed meal. The results of the writers' one trial reported above indicate, however, that cottonseed meal and linseed meal do not greatly differ in feeding value when fed to beef calves in combination with alfalfa hay, corn silage, and corn.

## DISCUSSION

When the data are considered in toto it is evident that not all species of animals respond alike when fed either linseed meal or cottonseed meal. Results with rats indicate that the proteins of these two feed stuffs are equally well digested, possess the same

<sup>5</sup> Unpublished data.

biological value, and are equally efficient in supplementing the proteins of corn, when fed on the same protein basis. Obviously, in the case of this species the two feeds would be of equal nutritive value. A review of the literature, on the other hand, reveals many discrepancies and conflicting results as to the relative nutritive value of linseed meal and cottonseed meal in livestock husbandry. If the variable results are due to an actual difference in the protein value of these feeds apparently similar differences should have been found in the case of the rat. Since no differences were noted, it is assumed that the discrepancy in the reported results may be explained, wholly or in part, on the basis of toxicity or species variation. This assumption is strengthened by the fact that the pigs and chicks in the writers' trials responded differently from rats or calves fed the same meals. In the case of the pigs the poor results with cottonseed meal can be explained upon the basis of toxicity. This explanation, however, will not account for the difference in response of the rats and chicks. With the last-named species the data indicate that the protein of cottonseed meal is superior to that of linseed meal for growth. Other unpublished data show that linseed meal may serve as a fairly efficient source of protein for egg production. This fact suggests that animals within the same species may react differently, depending upon age or a particular function. Obviously, not all species of animals are affected alike by the same food, and the question of how far the results of nutrition studies obtained with one species of animal are applicable to other species becomes a serious one. Investigation has shown that the vitamin requirements of different species vary. Likewise, the question of energy and mineral requirements between different species or a particular function must be given consideration. With some exceptions, a protein which proves of high biological value in case of the experimental rat is commonly considered of equal value for farm stock. While there is no evidence which would definitely show that animals differ in their amino acid requirements, the writers' data indicate that a difference in response to protein feeding may be observed. Whether this difference is one of protein digestion, utilization, or amino acid content remains to be determined. The final decision as to the quality or value of any feed must rest upon experiments with the species in question and with respect to a particular function such as growth, milk production, or egg production. Accordingly, feeders as well as investigators must exercise caution in attempting to interpret the nutrition of one species in terms of another.

#### SUMMARY

The proteins of linseed meal and cottonseed meal were equally well digested, possessed the same biological value, and were equally efficient in supplementing the proteins of corn, when fed on the same protein basis to young rats.

Rats and pigs did not respond alike when fed identical rations containing linseed meal and cottonseed meal. The latter proved toxic to pigs.

The feeding of linseed meal and cottonseed meal to growing chicks, either as the chief source of protein or in combination with an animal protein (meat scraps), showed that the proteins of cottonseed meal

were more efficient than those of linseed meal. No toxic effects were observed with this species.

Evidence is presented which indicates that the age or maturity of the chicken may influence its response to linseed-meal or cottonseed-meal feeding.

Cottonseed meal and linseed meal were of nearly equal value when fed to beef calves in combination with alfalfa hay, corn silage, and corn.

It is pointed out that not all species of animals respond alike to linseed-meal or cottonseed-meal feeding, and that the conflicting results obtained with these two feedings stuffs may, wholly or in part, be accounted for on the basis of toxicity or species variation.

#### LITERATURE CITED

- (1) ALMY, L. H., and ROBINSON, R. K.  
1920. TOXIC ACTION OF INGESTED LINSEED MEAL ON TROUT. *Jour. Biol. Chem.* 43: 97-111.
- (2) CULBERTSON, C. C., EVVARD, J. M., HAMMOND, W. E., and WALLACE, Q. W.  
1924. PROTEIN SUPPLEMENTS WITH DIFFERENT ROUGHAGES FOR FATTENING CATTLE. *Amer. Soc. Anim. Prod. Proc.* 1923: 13-24, illus.
- (3) DINWIDDIE, R. R.  
1903. PIG FEEDING EXPERIMENTS WITH COTTON SEED MEAL. *Ark. Agr. Expt. Sta. Bul.* 76, p. [133]-148.
- (4) FRAPS, G. S.  
1910. COTTONSEED MEAL AS HUMAN FOOD. *Tex. Agr. Expt. Sta. Bul.* 128, 15 p.
- (5) HART, E. B., and HUMPHREY, G. C.  
1917. THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. III. *Jour. Biol. Chem.* 31: 445-460, illus.
- (6) ——— and HUMPHREY, G. C.  
1918. THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. IV. *Jour. Biol. Chem.* 35: 367-383, illus.
- (7) HENRY, W. A., and MORRISON, F. B.  
1923. FEEDS AND FEEDING. Ed. 18, unabridged, 770 p., illus. Madison, Wis.
- (8) LINDSEY, J. B., BEALS, C. L., SMITH, P. H., and ARCHIBALD, J. G.  
1923. DIGESTION EXPERIMENTS WITH CATTLE FEEDS. *Mass. Agr. Expt. Sta. Bul.* 216, p. [53]-62.
- (9) MCCOLLUM, E. V.  
1922. THE NEWER KNOWLEDGE OF NUTRITION. Ed. 2, entirely rewritten, 449 p., illus. New York.
- (10) ——— and SIMMONDS, N.  
1917. A BIOLOGICAL ANALYSIS OF PELLAGRA-PRODUCING DIETS. II. THE MINIMUM REQUIREMENTS OF THE TWO UNIDENTIFIED DIETARY FACTORS FOR MAINTENANCE AS CONTRASTED WITH GROWTH. *Jour. Biol. Chem.* 32: 181-193, illus.
- (11) ——— and SIMMONDS, N.  
1917. A BIOLOGICAL ANALYSIS OF PELLAGRA-PRODUCING DIETS. III. THE VALUES OF SOME SEED PROTEINS FOR MAINTENANCE. *Jour. Biol. Chem.* 32: 347-368, illus.
- (12) ——— SIMMONDS, N., and PARSONS, H. T.  
1919. SUPPLEMENTARY RELATIONSHIPS BETWEEN THE PROTEINS OF CERTAIN SEEDS. *Jour. Biol. Chem.* 37: 155-178, illus.
- (13) MAYNARD, L. A., FRONDA, F. M., and CHEN, T. C.  
1923. THE PROTEIN EFFICIENCY OF COMBINATIONS OF CORNMEAL AND CERTAIN OTHER FEEDINGSTUFFS, NOTABLY RICE BRAN. *Jour. Biol. Chem.* 55: 145-155.
- (14) MENDEL, L. B., and FINE, M. S.  
1912. STUDIES IN NUTRITION. V. THE UTILIZATION OF THE PROTEINS OF COTTON SEED. *Jour. Biol. Chem.* 11: 1-3.

- (15) MITCHELL, H. H.  
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. *Jour. Biol. Chem.* 58: 873-903.
- (16) ——— BEADLES, J. R., and KEITH, M. H.  
1926. THE VALUE OF COCOA AND CHOCOLATE AS SOURCES OF PROTEIN IN THE DIET. *Jour. Biol. Chem.* 71: 15-31.
- (17) NEVENS, W. B.  
1921. THE PROTEINS OF COTTONSEED MEAL. II. NUTRITIVE VALUE. *Jour. Daily Sci.* 4: 552-588.
- (18) OSBORNE, T. B., and MENDEL, L. B.  
1916. THE EFFECT OF THE AMINO-ACID CONTENT OF THE DIET ON THE GROWTH OF CHICKENS. *Jour. Biol. Chem.* 26: 293-300, illus.
- (19) ——— and MENDEL, L. B.  
1917. THE USE OF COTTON SEED AS FOOD. *Jour. Biol. Chem.* 29: 289-317, illus.
- (20) ——— and MENDEL, L. B.  
1917. THE RELATIVE VALUE OF CERTAIN PROTEINS AND PROTEIN CONCENTRATES AS SUPPLEMENTS TO CORN GLUTEN. *Jour. Biol. Chem.* 29: 69-92, illus.
- (21) ——— and MENDEL, L. B.  
1918. NUTRITIVE FACTORS IN PLANT TISSUES. I. THE PROTEIN FACTOR IN THE SEEDS OF CEREALS. *Jour. Biol. Chem.* 34: 521-535, illus.
- (22) ——— MENDEL, L. B., and FERRY, E. L.  
1919. A METHOD OF EXPRESSING NUMERICALLY THE GROWTH-PROMOTING VALUE OF PROTEINS. *Jour. Biol. Chem.* 37: 223-229.
- (23) RATHER, J. B.  
1913. DIGESTION EXPERIMENTS ON MEN WITH COTTONSEED MEAL. *Tex. Agr. Expt. Sta. Bul.* 163, 26 p.
- (24) RICHARDSON, A. E., and GREEN, H. S.  
1916. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. I. *Jour. Biol. Chem.* 25: 307-318, illus.
- (25) ——— and GREEN, H. S.  
1917. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. II. *Jour. Biol. Chem.* 30: 243-258, illus.
- (26) ——— and GREEN, H. S.  
1917. NUTRITION INVESTIGATIONS UPON COTTONSEED MEAL. III. COTTONSEED FLOUR. THE NATURE OF ITS GROWTH-PROMOTING SUBSTANCES, AND A STUDY IN PROTEIN MINIMUM. *Jour. Biol. Chem.* 31: 379-388, illus.
- (27) SCHWARTZ, E. W., and ALSBERG, C. L.  
1924. RELATION BETWEEN TOXICITY OF COTTONSEED AND ITS GOSSYPOL CONTENT. *Jour. Agr. Research* 28: 173-189, illus.
- (28) SMITH, H. R.  
1907. ECONOMICAL RATINGS IN BEEF PRODUCTION. *Nebr. Agr. Expt. Sta. Bul.* 100, 40 p., illus.
- (29) TOMHAVE, W. H., and BENTLEY, F. L.  
1923. STEER FEEDING EXPERIMENTS AT THE PENNSYLVANIA STATE COLLEGE. *Penn. Agr. Expt. Sta. Bul.* 183, 16 p., illus.
- (30) WITHERS, W. A., and BREWSTER, J. F.  
1913. STUDIES ON COTTON SEED MEAL TOXICITY. II. IRON AS AN ANTIDOTE. *Jour. Biol. Chem.* 15: 161-166.
- (31) ——— and CARRUTH, F. E.  
1915. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL. *Jour. Agr. Research* 5: 261-288, illus.
- (32) ——— and CARRUTH, F. E.  
1917. IRON AS AN ANTIDOTE TO COTTONSEED MEAL INJURY. *Jour. Biol. Chem.* 32: 245-257, illus.
- (33) ——— and CARRUTH, F. E.  
1918. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED. *Jour. Agr. Research* 12: 83-102, illus.



# CORRELATED INHERITANCE IN KANRED $\times$ SEVIER VARIETIES OF WHEAT<sup>1</sup>

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## INTRODUCTION

In a previous study<sup>2</sup> the writer made a critical analysis of segregates from the wheat cross Sevier  $\times$  Federation. The data indicated a single major factor and a series of minor modifying factors for the inheritance of head density. Both the parents used in this cross were of intermediate head density, but some of the F<sub>3</sub> progenies had heads much more dense, and others had heads much more lax, than those of either parent. Segregates with the head density of Federation, the less dense of the two parents, were recovered in a few cases, but out of 321 F<sub>3</sub> progenies studied only 1 had a head density that even approached that of Sevier, and it was far from certain statistically that the density of Sevier was recovered in this segregate. It is more accurate to say that this was the only one of the 321 F<sub>3</sub> families studied which was not definitely different statistically from the Sevier parent in head density.

Awn inheritance also showed a behavior not hitherto observed. Federation was awnless in the usual sense, while Sevier was fully awned. Some homozygous F<sub>3</sub> families were obtained similar to one parent and some similar to the other parent; two intermediate forms of partly awned homozygous segregates were also obtained but in regularly smaller numbers than in the parental types. This and the ratios in the segregating types led to the suggestion of two factors for awns so linked as to produce gametes<sup>3</sup> in the ratio 1.8:1:1:1.8. Good fits by the X<sup>2</sup> method were obtained in two families and not a bad fit in a third family.

Simple correlation coefficients ( $r$ ) and correlation ratios ( $\eta$ ) were calculated for a number of characters. It was concluded that:

In view of the consistently significant correlations, as judged by their probable errors, between the two stable and definitely inherited characters of spike density and awn classes, it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters.

The cross Kanred  $\times$  Sevier, made previously for the purpose of securing better winter wheats, fortunately involved just the right characters for a careful testing of the suggested linkage between the factors for awns and those for spike density.

The following study is therefore a by-product of an economic plant-breeding project. Not many data were taken in F<sub>2</sub>, but since every F<sub>2</sub> plant was represented by an F<sub>3</sub> progeny no trouble was

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<sup>2</sup> STEWART, G. CORRELATED INHERITANCE IN WHEAT. Jour. Agr. Research 33: 1163-1192, illus. 1926.

<sup>3</sup> STEWART, G. INHERITANCE OF AWNS IN CROSSES INVOLVING SEVIER AND FEDERATION WHEATS. Jour. Amer. Soc. Agron. 20: 160-170. 1928.

encountered in making the genetic studies required to test the correlation previously suggested. The number of parent rows was, however, fewer than would have been the case had the test been definitely planned for correlation studies. Nevertheless, there were enough of these for reasonable comparison.

## EXPERIMENTAL DATA

### SCOPE OF THE INVESTIGATION

The investigational work here reported has to do with—

1. Inheritance studies of (1) spike density, (2) awn length, (3) plant height, (4) number of spikelets, (5) number of culms, (6) grain color, and (7) glume color.
2. Correlation between head density and awn length.
3. Simple, partial, and multiple correlations between the following characters: (1) Spike density, (2) awn length, (3) plant height, (4) number of spikelets, and (5) number of culms.

### DESCRIPTION OF PARENTS

The parents used were Kanred and Sevier (pure line No. 59), a variety recently discovered<sup>4</sup> in Sevier County, Utah. So far as the writer is aware, this is the only region where Sevier wheat is grown commercially, though it has been recently introduced into the upper Snake River Valley of Idaho.

#### SEVIER

The variety Sevier has commercial importance in the Sevier River Valley of Utah, where occasionally there is some black stem rust. The straw is extremely weak, and the grain lodges badly, although unless lodging is extremely severe there is little loss, for Sevier seems to have a certain amount of resistance to the physiologic forms of *Puccinia graminis tritici* that occur in this region. It is extremely high yielding under favorable conditions, is somewhat drought resistant, and is also thought to be slightly resistant to alkali, though this has not been proved. The spike is awned and is somewhat laterally compressed, and though considerably more dense of spike than is Kanred, Sevier can not be classed as a club wheat. The glumes are bronze but not dark bronze. The kernels are white, and in some pure lines are almost as hard as those of durum wheats, whereas in others the kernels are soft. In the pure line used as a parent of the hybrids studied the kernels are hard but not so hard as in some of the other lines. The grain is held firmly in the chaff and no amount of weathering seems to cause shattering, a fact which makes it a desirable parent to use in spring-wheat crosses both with Federation and Dicklow, as these two varieties both shatter considerably, and with Turkey or Kanred, which shatter slightly if left standing long after cutting readiness.

#### KANRED

The Kanred variety resulted from a pure-line selection from Crimean wheat at the Kansas station. It resembles ordinary Turkey rather closely, but differs from it visibly in having appreciably longer beaks on the glumes. Kanred is very important in the central Great Plains and has recently increased rapidly in the dry-farming sections

<sup>4</sup> STEWART, G. SEVIER WHEAT. Jour. Amer. Soc. Agron. 15: 385-392. 1923.



of the central Rocky Mountain region. It is known to be immune to 11 of the common physiologic races of black-stem rust and to be more drought resistant in the central Great Plains than is common Turkey. In parts of Utah it has given higher acre yields than Turkey, otherwise the best commercial variety for the region, and is thought to be somewhat more winter hardy. It has the winter growth habit and is fully awned. It has a lax to mid-dense fusiform spike and a hard dark red kernel. The straw is considerably shorter than that of Sevier, but is nearly, though not quite so much, inclined to lodge. The chaff is white with faint bronze markings.

#### GENETIC STUDY

The very promising yield obtained from various segregates of crosses in which Sevier was one of the parents, and the frequent occurrence of recombinations of genetic factors<sup>5</sup> whereby segregates were obtained which were more resistant to *Puccinia graminis tritici* than either parent, led to an attempt to secure valuable wheat strains from the cross of Sevier on Kanred, which is probably the best commercial winter wheat of the region.

The cross was made at Logan, Utah, during the summer of 1923, between a pure line of Kanred and pure line No. 59 of Sevier. About 20 F<sub>1</sub> kernels were obtained and all were seeded that fall about 1 foot apart each way in a well-fertilized seed bed. The F<sub>1</sub> plants grew vigorously and yielded abundantly, producing from 800 to 2,500 kernels each, only a part of which were sown. Ten of the F<sub>1</sub> plants were harvested and grains from them seeded in the fall of 1924. The plants were spaced about 4 inches in rows 1 foot apart. The F<sub>2</sub> plants were studied from the standpoint of straw strength. Just before harvest two families which seemed to stand up better were selected for further study. The two F<sub>2</sub> families selected bore the pedigree numbers 15a-4 and 15a-7. The plants of each family were classified roughly as to head density and color of chaff, and accurately as to grain color. In the fall of 1925 an F<sub>3</sub>-progeny row was seeded with the grain of each F<sub>2</sub> plant. There were seeded about 40 kernels in each F<sub>3</sub>-progeny row. The rows were 1 foot apart, and the kernels were spaced 3 to 4 inches apart in the row. The data were taken on the F<sub>3</sub>-progeny rows at harvest in July and August, 1926.

#### SPIKE DENSITY

The F<sub>1</sub> plants had spikes that were of intermediate density, somewhat like those of the Sevier parent. No measurements, however, were taken in this case either of the F<sub>1</sub> or of the F<sub>2</sub> plants. In F<sub>2</sub> there was a notable transgressive segregation, some plants having considerably more dense spikes than either parent. The extremely lax spikes were not far different from the most lax spikes of the more lax parent, Kanred. In F<sub>3</sub> the density of each plant in each progeny was measured, and the mean of the 30 or 40 plants in each progeny used as the true classification of the F<sub>2</sub> plant from which it was descended.

The spike density was obtained by measuring carefully on a leading spike of each plant the length of 10 internodes along the central part of the rachis. The rachis internodes at the extreme base of the spike

<sup>5</sup> STEWART, G. ORIGIN OF A SEGREGATE RESISTANT TO BLACK-STEM RUST IN A CROSS BETWEEN TWO SUSCEPTIBLE PLANTS. *Amer. Nat.* 62: 188-191. 1928.

and those at the extreme apex of the spike were avoided for the reason that they often vary both considerably and irregularly from those more centrally located. Measurement began, therefore, with the third or fourth spikelet internode from the bottom of the spike. It was found most convenient to take the measurement from the base of the third or fourth spikelet internode to the base of the sixth internode above on the same side of the spike. This gave the length of 10 internodes. When the mean length of 10 internodes for an  $F_3$ -progeny row was calculated, the mean length of each spikelet internode if desired was secured merely by pointing off one decimal place.

Not only was the mean density of each progeny obtained, but the coefficient of variability for each individual progeny was calculated. In Table 1, the spike densities of the parental rows of Sevier 59 and of Kanred and of the  $F_3$  progenies are given. The  $F_3$  progenies are arranged into three groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes. Spike density classes in millimeters to the spikelet internode are shown at the top of the table. On the extreme right the coefficients of variability classes (C. V.) are designated.

TABLE 1.—*Spike-density classes of the means of Sevier and Kanred parental rows and of the means of  $F_3$  progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of Sevier and Kanred parents and of three groups of  $F_3$  hybrid progenies: (1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a-7; grown in 1926 at Petersburg, Utah]

Strain	Number of plants in spike-density classes (millimeters)								Total	C. V. classes	
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25			5.75
Sevier 59.....	{		1	6						17	
Total.....			1	7						Mean.	8.46
Kanred.....	{							2	2	6	6
								5	1	6	9
											12
								2		2	15
Total.....								9	1	Mean.	9.46
Homozygous dense.....	{	4	23							4	6
		1	11							27	9
			1							12	12
		1								1	15
										1	18
Total.....		6	39							Mean.	9.83
Heterozygous.....	{			3	1					1	21
				12	3	2				4	24
			3	15	8	1				20	27
			4	5	5					28	30
			5	17	5	1				27	33
			1	6	3					10	36
			6	1						7	39
			1	1						2	42
			1		1					2	45
											48
				1						1	51
Total.....			21	56	22	3				Mean.	31.82
Homozygous lax.....	{					8	2			10	6
						5	9	8		22	9
							4	2	1	7	12
Total.....						5	21	12	1	Mean.	10.45

Table 1 proves that there were progenies which were homozygous for dense spikes and others homozygous for lax spikes, whereas the plants of intermediate spike densities were all heterozygous. The coefficients of variability were used to indicate homozygosity or heterozygosity. The mean coefficients of variability for Sevier 59 and for Kanred were 8.46 and 9.46 per cent, respectively. An  $F_3$  progeny was regarded as homozygous when its coefficients of variability were not essentially greater than those of the parental rows. Sevier 59 had a range of 6.6 to 9.8 per cent. The range for Kanred was from 6 to 16.4 per cent.

In the homozygous dense  $F_3$  progenies the range in coefficients of variability was from 6.5 to 18 per cent. Only two were higher than 13.5 per cent. The mean was 9.83 per cent. In the homozygous lax group the range was from 6 to 13 per cent with a mean of 10.45 per cent.

In the heterozygous progenies, the coefficients of variability are so high as to prove heterozygosity at a glance. The range is from 22 to 50 per cent with a mean of 31.82 per cent. While there was no extreme gap between the most variable progeny classed as homozygous dense (C. V.=18 per cent) and the least variable progeny classed as heterozygous (C. V.=22 per cent), there was evidence in the material itself that they did not belong in the same groups. At most only this one, the most variable, of the homozygous dense progenies could be questioned.

Examination of Table 1 also proves that there was transgressive segregation in the direction of greater spike density. There is not one of the homozygous dense progenies that is not more dense than the most dense row of the more dense parent, Sevier 59. The most dense row of Sevier had a mean spike density of 2.983 mm. to the spikelet internode, whereas the most lax row of the homozygous dense progenies was 2.488 mm. The number and distribution of the parent rows did not permit the measurement of the variability of the soil, but it is extremely doubtful that there was even one homozygous dense progeny that approximates Sevier in head density. Not only was the segregation in this respect transgressive, but it was so strikingly so that all homozygous dense progenies were appreciably more dense than the most dense row of Sevier.

The homozygous lax progenies were very similar in spike density to the rows of the more lax parent, Kanred. Some of the more compact progenies in this group were considerably more compact than the most compact row of Kanred. Transgressive segregation did not in this cross occur at the lax end of the segregation as it did in the previous cross between Sevier × Federation.

The corresponding data for family 15a-4 are given in Table 2. There is no real difference in any respect save that three  $F_3$  progenies in the homozygous dense group were slightly less dense than any in family 15a-7. In the progenies breeding true for dense spikes the coefficients of variability ranged from 7.6 to 14.5 per cent, with a mean 10.91 per cent. Those homozygous for lax spikes had a mean coefficient of variability of 7.71 per cent, with a range of from 5.7 to 11.5 per cent. On the other hand, the coefficients of variability of the progenies heterozygous for spike density ranged from 20.9 to 43.2 per cent, with a mean of 31.1 per cent. As in family 15a-7, it was easy to distinguish the true-breeding progenies from the segregating ones by the size of the coefficient of variability.

TABLE 2.—*Spike-density classes of the means of Sevier and Kanred parental rows and of the means of  $F_3$  progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of Sevier and Kanred parents, and of three groups of  $F_3$  hybrid progenies: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a-4; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in spike density classes (millimeters)								Total	C. V. classes
	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75		
Sevier 59		1	6						7	6
Total									Mean.	8.46
Kanred							2	1	3	6
							5		5	9
									0	12
							2		2	15
Total							9	1	Mean.	9.46
Homozygous dense	1								1	6
	4	1							5	9
	11	1							12	12
	1	1							2	15
Total	17	3							Mean.	10.91
Heterozygous	1		1						1	21
			3	3					1	24
		2	7	2					6	27
		3	4	2					11	30
		1	5						9	33
		1							6	36
			1						1	39
									1	42
Total	1	7	21	7					Mean.	31.10
Homozygous lax						6	8		14	6
						2	2		4	9
						2		1	3	12
Total						10	10	1	Mean.	7.71

In family 15a-4, the least dense progeny had a mean spike density of 2.723 mm. to the spikelet internode. As compared with family 15a-7, this is considerably nearer the most dense row of Sevier, which had a mean density 2.983 mm. The next least dense  $F_3$  progeny in this group was 2.511 mm., which is almost identical with the least dense one in family 15a-7. It can not be said, therefore, with any real assurance that in any true-breeding progeny in this cross was the density of the Sevier parent recovered. On the other hand, it could not be proved that this one least dense progeny with a mean density of 2.723 mm. was statistically less dense than the most dense row of Sevier—2.983 mm.

In family 15a-7 there are 187  $F_3$  progenies, of which 45 bred true for dense spikes, 39 bred true for lax spikes, and 102 segregated for spike density. This suggests a 1:2:1 segregation—that is, a difference of one factor. The calculation for closeness of fit on this hypothesis is given in Table 3.

TABLE 3.—Closeness of fit of three groups of  $F_3$  progenies on a 1:2:1 segregation

[Family 15a-7, grown in 1926 at Petersburg, Utah]

Group	C	O	C-O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense.....	45	46.5	-1.5	2.25	0.0500
Heterozygous.....	102	93.0	9.0	81.00	.8710
Homozygous lax.....	39	46.5	-7.5	56.25	1.2097
$X^2=2.1291 \quad P=0.3478$					

In family 15a-4 there were 77  $F_3$  progenies, of which 20 bred true for dense spikes, 21 for lax, and 36 segregated. The calculations for closeness of fit on a 1:2:1 segregation are given in Table 4.

TABLE 4.—Closeness of fit of three groups of  $F_3$  progenies on a 1:2:1 segregation

[Family 15a-4, grown in 1926 at Petersburg, Utah]

Group	C	O	C-O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense.....	20	19.25	0.75	0.5625	0.0292
Heterozygous.....	36	39.50	2.50	6.2500	.1623
Homozygous lax.....	21	19.25	1.75	3.0625	.1591
$X^2=0.3506 \quad P=\text{very high}$					

Since  $X^2$  is less than 1, the probability is very high that the hypothesis is at least approximately correct. In family 15a-7,  $P=0.3478$ —that is, in 35 cases out of 100 a worse fit might be expected due to chance alone. There also is a high probability that the 1:2:1 hypothesis fits the facts rather closely.

There seems, therefore, to be a genetic difference of one major factor for head density involved in this cross.

Table 5 proves that there is considerably more variability in spike density, as measured by the coefficient of variability both in the homozygous dense progenies and in the homozygous lax progenies, than in either parent. The coefficient of variability (C. V.) for the Sevier rows was 3.7 per cent and for the Kanred rows 2.83 per cent. In family 15a-7 the coefficients of variability for homozygous dense and for the homozygous lax progenies were 7.78 and 5.62 per cent, respectively. In family 15a-4 coefficients of variability for the homozygous dense and for the homozygous lax groups were 6.32 and 4.91 per cent. The same figures for the heterozygous groups in families 15a-7 and 15a-4 were 9.91 and 10.60 per cent, respectively.

TABLE 5.—*The range of mean spike densities and the mean of mean spike densities of Sevier and Kanred rows, and of three groups of  $F_3$  progenies, together with the coefficients of variability (C. V.) of the means of Sevier and Kanred rows and of the  $F_3$  progenies*

[Families 15a-7 and 15a-4; grown in 1926 at Petersboro, Utah]

Strain	Range of spike densities of parental rows and of $F_3$ progenies		Mean of spike densities of parental rows and $F_3$ progenies		C. V. of mean spike densities of parents and $F_3$ progenies	
	Family 15a-7	Family 15a-4	Family 15a-7	Family 15a-4	Family 15a-7	Family 15a-4
Sevier 59.....	2.983-3.375	2.983-3.375	3.199	3.199	3.70	3.70
Kanred.....	5.127-5.632	5.127-5.632	5.292	5.292	2.83	2.83
Homozygous dense.....	1.753-2.488	2.015-2.723	2.134	2.361	7.78	6.32
Heterozygous.....	2.615-4.121	2.852-4.045	3.265	3.250	9.91	10.60
Homozygous lax.....	4.445-5.533	4.696-5.688	4.854	5.080	5.62	4.91

Since the variability in the true-breeding progenies is about double that found in similar rows of parental material, it is apparent that there must be present, in addition to the single major factor for spike density, some minor factors which bring this about.

#### AWN LENGTH

Since both parents were fully awned, it is unlikely that any attention would have been given to this character had there not been the strong indication already noted of correlation between awn length and spike density. As it was, no data were taken in either  $F_1$  or  $F_2$ . However, in  $F_2$  general observations were made. It was thought that the compact heads bore somewhat regularly shorter awns than did the lax heads.

In the summer of 1926 the awns of each plant in each of the progenies were measured. The mean length of the 30 or 40 plants was used as the correct figure for the parental  $F_2$  plant. The coefficient of variability for each individual progeny was then calculated. In order to ascertain whether the anticipated correlation between awn length and spike density really existed, correlation coefficients ( $r$ ) and other correlation constants were calculated, with the results reported in a later section of this paper. Since a high correlation was found, the awn-length data given in Table 6 are classified into the three principal spike-density groups in which the progenies of given awn lengths were found. Even the gross grouping so made discloses the presence of a considerable correlation. The approximate centers of the progeny distribution for awn length in the groups which had dense spikes, which was heterozygous for spike density, and which had lax spikes, respectively, roughly arrange themselves in a diagonal line.

Table 6 also establishes clearly that Sevier 59 has much shorter awns than Kanred. The awns of Sevier are so appreciably shorter that there is a considerable gap between the Sevier row with the greatest mean length and the Kanred row with the least mean length. The  $F_3$  progenies show a distinct segregation which yields progenies throughout almost the entire range between that row of Sevier with the shortest awn length to that row of Kanred with the greatest.

Segregation was not of such a nature that ratios between the various classes were obtained. With carefully grouped, replicated, and checked plantings it is likely that this could be done. However, the wide variation in awn length, as indicated by large standard deviations, would make this task one that called for refined statistical methods, both in the planting plan and in taking and calculating the data.

TABLE 6.—*Awn-length classes of the means of Sevier and Kanred parental rows and of the means of the F<sub>3</sub> progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of the Sevier and Kanred parents and of the F<sub>3</sub> hybrid progenies. In order to show the close correlation between spike density and awn length, the F<sub>3</sub> hybrid progenies are grouped according to the three spike-density groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a-7; grown in 1926 at Petersburg, Utah]

Strain	Number of plants in awn-length classes (millimeters)																Total	C. V. classes
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96		
Sevier 59						1											1	6
					2	1											3	9
				1	2												3	12
																		15
																		18
	1																1	21
Total	1			1	4	2											Mean.	13.48
Kanred											1	1	3		1	1	3	12
											1						5	15
																	1	18
										1								21
																	1	24
Total										1	2	2	3		1	1	Mean.	15.65
Homozygous dense	1		2	3	7	3	1	2	4	2							8	9
				2	3	1			1								27	12
					1												8	16
						1											1	18
																	1	21
Total	1		2	5	12	10	3	9	3								Mean.	12.56
Heterozygous							3	4	2	3	1			1			1	9
							8	8	11	7	3						14	12
				1	4	2	7	7	6	3	1					1	47	15
					1		2	4	1	1							31	18
																	9	21
Total				1	5	13	22	20	21	14	5				1	1	Mean.	16.02
Homozygous lax								1	1	1	3	1		3			10	12
								3	5	3	3	3		1	1		22	15
								1		1	1	2			1		7	18
Total							2	4	6	5	7	5	4	4	2		Mean.	14.61

Similar data for family 15a-4 are given in Table 7. This table is as near an exact duplicate of Table 6 as could be hoped for. The only difference is that the awns of the F<sub>3</sub> progeny with the greatest mean awn length is 6 mm. shorter than the corresponding one in family 15a-7. (Table 6.) This is not surprising, however, in view of the fact that this family had in it less than half as many F<sub>3</sub> progenies, and also in view of the fact that it was grown on less productive land. Here the two Kanred rows with the greatest mean

awn length stand well out beyond the  $F_3$  progeny having the greatest mean awn length. The defect of having too few parental rows prevents a definite conclusion that the  $F_3$  progenies really lacked some of the awn length of the Kanred parent.

TABLE 7.—Awn-length classes of the means of Sevier and Kanred parental rows and of the means of the  $F_3$  progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of the Sevier and Kanred parents and of the  $F_3$  hybrid progenies. In order to show the close correlation between spike density and awn length, the  $F_3$  hybrid progenies are grouped according to the three spike-density groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes

[Family 15a-4; grown in 1926 at Petersburg, Utah]

Strain	Number of plants in awn-length classes (millimeters)																Total	C. V. classes
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96		
Sevier 59						1											1	6
						2	1										3	9
				1	2												3	12
																		15
	1																1	18
Total	1			1	4	2											Mean	13.48
Kanred											1	1			1	1	3	12
											1	1	3				5	15
																	1	18
																		21
											1						1	24
Total											1	2	2	3		1	Mean	15.65
Homozygous dense			1	2	5	2	1	1									12	12
					1	2	1										4	15
			1		1												2	18
	1			1													2	21
Total	1		2	3	7	4	2	1									Mean	14.16
Heterozygous		1		2	5	2	4	2	2	2	1						4	12
					2	1			1								24	15
				1	1												2	18
					1												1	21
																		24
Total		1		3	9	6	4	5	3	4	1						Mean	15.73
Homozygous lax								2	3	2	1	4	2				1	9
									1		2		1				13	12
																	4	15
						1											1	18
																	1	21
Total						1		2	4	3	4	4	3				Mean	14.02

#### HEIGHT OF PLANT

Height of plant was obtained by measuring each of the 30 or 40 plants in each parental row and in each  $F_3$  progeny. The roots of the pulled plant were pressed firmly against a footboard nailed perpendicularly to a baseboard which was marked in centimeters. The height of the plant was read directly, to the base of the spike on the longest culm. It might have been more accurate, therefore, to have designated this character as the length of the longest culm. As with the other characters, the mean for the row was the figure used.



The shortest row of Kanred was 75.9 cm. tall and the tallest row 101.5 cm.; the mean height was 89.2 cm. The mean height of the shortest row of Sevier 59 was 94.9 cm., of the tallest row 113.1 cm., and the mean of all rows 102.6 cm. The height of the shortest  $F_3$  progeny was 87.9 cm., the tallest one was 126.7 cm., and the mean of all was 107.1 cm. No  $F_3$  progenies were obtained as short as the shortest Kanred row, but the tallest  $F_3$  progeny exceeded the tallest Sevier row by almost as many centimeters as the shortest  $F_3$  progeny exceeds the shortest Kanred row. There were too few parental rows to permit very accurate conclusions. While from casual examination of Table 8 it looks as if there were transgressive segregation in at least the tallest progeny, which was 13.6 cm. taller than the tallest row of Sevier, this difference is not much greater than the probable error.

TABLE 8.—Frequency distribution of the rows of the Sevier and Kanred parents and of 187  $F_3$  hybrid progenies, arranged into classes according to mean height of the tallest culm and according to standard deviation classes (S. D.) of the individual rows of parents and of  $F_3$  progenies

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in plant-height classes (centimeters)																	Total	S. D. classes
	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120	123	126	
Sevier 59								1	1	1		1	1	1					6
								1											1
								1											1
Total								3	1	1		1	1	1					Mean.
Kanred	1	1	1			1													3
								1	1	1									6
								1											9
										1									2
Total	1	2	1			1	1	2	1	1									Mean.
$F_3$ progenies					1	4	5	12	12	14	18	15	11	1					92
						7	10	7	15	13	13	5	3	1	1				76
						1		3	2	2	1	1	1						11
									1										2
											1				1	1			15
												1	1				1		18
																		1	4
																			1
Total					1	12	15	22	30	29	33	22	16	2	2	1		1	Mean.

The distribution of the mean heights of the parental rows and of the  $F_3$  progenies in classes, according to height and according to standard deviation (S. D.), is shown in Table 8. The frequency chart in this table shows a genetic difference in height between the two parents. The distribution of the mean heights of the  $F_3$ -progeny rows indicates a segregation for height. The nature of the segregation could not be accurately determined from the data secured.

#### NUMBER OF SPIKELETS

The number of spikelets was obtained by actual count, the sterile spikelets not being included. This set of data was taken from the same leading spike used for spike-density and for awn-length measurements. As in the other cases, the mean for the 30 or 40 plants in each parent row or in each progeny was the figure used.

The smallest number of spikelets to the spike on any row of Sevier 59 was 12.83; the highest number was 15.85; the mean was 14.78. For Kanred the lowest, highest, and mean numbers were 13.60, 17.25, and 14.45, respectively. In the  $F_3$  progenies the lowest number of spikelets to the spike was 11.84 and the highest number 18.15. The mean was 15.77. Although the mean number of spikelets is higher in the  $F_3$  progenies it is not significantly so, the difference being about one times the probable error.

There was, however, one  $F_3$  progeny which had a smaller mean number of spikelets than any parental row and one which had more than any parental row. In Table 9, the distribution, according to spikelet-number classes arranged also in standard deviation classes (S. D.), brings out this apparent transgressive segregation. Two more classes are required at the higher end of the distribution for the  $F_3$  progenies than for the highest parental row, which itself was three classes higher than the next highest parental row. The amount of variability does not differ materially in the two parents and in the  $F_3$  progenies.

TABLE 9.—Frequency distribution of the rows of the Sevier and Kanred parents and of 187  $F_3$  hybrid progenies, arranged into classes according to mean number of spikelets per leading spike of each plant and according to standard deviation classes (S. D.) of the individual rows of parents and of  $F_3$  hybrid progenies

[Family 15a-7; grown in 1926 at Petersburg, Utah]

Strain	Number of plants in spikelet classes												Total	S. D. classes
	12.75	13.25	13.75	14.25	14.75	15.25	15.75	16.25	16.75	17.25	17.75	18.25		
Sevier 59	1		1	1	1	1	2						17	12
Total	1		1	1	1	1	3						Mean.	1.91
Kanred			1	2		1				1			17	12
Total			4	3		1				1			Mean.	1.99
$F_3$ families			1	5	20	29	24	16	22	7	6		135	23
			1	4	4	4	9	8	4	4	1		39	3
													1	4
Total			9	9	24	35	33	28	28	12	6		Mean.	2.12

There seems to be a segregation in the number of spikelets, though its nature could not be determined from the data available. The data indicate that very careful statistical work would be required to obtain the nature of the segregation.

#### NUMBER OF CULMS

The number of culms to the plant was obtained by actual count on all of the plants in each parental row and in each  $F_3$  progeny. The mean for each row was used as representing properly the parent  $F_2$  plant.

The mean number of culms per plant ranged from 8.97 to 12.12 in the rows of Sevier 59, and from 7.82 to 13.45 in the rows of Kanred.

The means were 10.12 and 10.43 for Sevier 59 and Kanred, respectively. In the  $F_3$  progenies the range was from 7.40 to 14.89, and the mean was 10.56 culms per plant.

Table 10 shows the parental rows and the  $F_3$  progenies grouped into classes for the mean number of culms and for the size of the standard deviations (S. D.). There seems to be a slight indication of transgressive segregation in the direction of a few rows with more culms to the plant. The mean standard deviation (S. D.), however, is no greater for the  $F_3$  progenies than for the parents. It may be that the indication of transgressive segregation is only apparent, since the small number of parental rows probably does not show the entire range in their respective variabilities.

TABLE 10.—Frequency distribution of the rows of the Sevier and Kanred parents and of 187  $F_3$  hybrid progenies, arranged into classes according to mean number of culms per plant and according to size of the standard deviation (S. D.) of the individual rows of parents and  $F_3$  hybrid progenies

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in culm number classes										Total	S. D. classes
	7	8	9	10	11	12	13	14	15			
Sevier 59.....	{		1								1	3
			2	1	1						4	4
			2			1					3	5
Total.....			5	1	1	1					Mean.	4.25
Kanred.....	{		1								1	3
			1	1		2					4	4
					1	1	1				2	5
		1		1			1				3	6
Total.....		1	2	1	1	3	1	1			Mean.	4.73
F <sub>2</sub> progenies.....	{	2	11	12	2	9	8	1			2	2
		3	8	21	17	14	8	2	1		43	3
			2	10	11	13	8	3	2		74	4
					2	3	3		1		49	5
				1	2		3				9	6
						1	1				3	7
					1					1		8
					1						2	9
				1						1	10	
				2						2	11	
					1					1	12	
Total.....		5	21	44	46	39	21	5	4	1	Mean.	4.37

#### COLOR OF GRAIN

In 1926 each  $F_3$  plant was classified as to grain color. The progenies were then grouped into true-breeding reds, true-breeding whites, and those segregating for red and white. The preliminary grouping indicated the likelihood of the three-factor differences for grain color. This led to a study of the nature of the segregation in each of the segregating rows. The calculated expectancy for each 64 plants on a three-factor basis is as follows:

True-breeding red grain.....	37
Segregating 63 red : 1 white.....	8
Segregating 15 red : 1 white.....	12
Segregating 3 red : 1 white.....	6
True-breeding white grain.....	1

Examination of the data showed an excess of true breeding reds and a deficiency in the segregating classes. Since there were only 30 to 40 plants in each progeny, there would be several that should segregate 63 : 1, which would fail to show the one plant with white grain. In the fall of 1926, when  $F_4$  progenies were seeded in order to get pure lines for rod-row testing, the rows were made long enough to include from about 100 to 110 plants. Altogether, 957  $F_4$  progenies were grown. Several of these came from each of 56 progenies which were true for red grain in 1926. This gave a good check on these 56 rows. Of these, 49 bred true for red grain; 6 segregated as 63 red : 1 white; and 1 segregated as 15 red : 1 white. In addition, two progenies which were intermediate between 63 : 1 and 15 : 1 proved to be segregating for 15 red : 1 white. The  $F_3$  data on three other progenies left doubt as to whether they were segregating 15 : 1 or 3 : 1. Two of these proved to be 3 : 1 and one 15 : 1. Since only about half the  $F_3$  progenies were tested, it would have been legitimate to double these corrections. This would have given an extremely close fit to the expected numbers in each class. However, in order to avoid all criticism, only the actually observed corrections were made. The results so obtained are given in Table 11.

TABLE 11.—Number of  $F_3$  progenies (1926) in each class based on 40 plants to the row, and the number of corrections obtained in  $F_4$  (1927) on the basis of about 100 plants to the row

[Family 15a-7; grown at Petersboro, Utah]

Class as to grain color	Number of $F_3$ progenies	$F_4$ corrections	Final count
True-breeding red.....	127	.....	120
63 red : 1 white.....	* 121 (?)	{ 6 4 2	18
15 red : 1 white.....	24		28
3 red : 1 white.....	* 15 (?)		17
True-breeding white.....	3	.....	3
	186	.....	186

\* Intermediate. Not sure as to classification.

Table 12 shows the closeness of fit for the color-of-grain data on the basis of a three-factor difference between the two parents.

TABLE 12.—Closeness of fit of five groups as to grain color on a three-factor difference (37:8:12:6:1 ratio)

[Family 15a-7; grown in 1926 and 1927 at Petersboro, Utah]

Group	C	O	C-O	(C-O) <sup>2</sup>	$\frac{(C-O)^2}{C}$
Homozygous red grain.....	107.53	120	-12.47	155.5009	1.4461
Segregating 63 red : 1 white.....	23.25	18	5.25	27.5625	1.1855
Segregating 15 red : 1 white.....	34.88	28	6.88	47.3344	1.3571
Segregating 3 red : 1 white.....	17.44	17	.44	.1936	.0111
Homozygous white grain.....	2.91	3	-.09	.0081	.0028
$X^2=4.0001 \quad P=0.4060$					

It seems reasonable to conclude that the theory of a three-factor difference fits the facts, since a  $P$  of 0.41 reasonably approximates

the desired 0.50. If the corrections obtained on half the  $F_3$  progenies were assumed to apply to the other half,  $P$  would then become 0.78. It is to be emphasized, however, that no such assumptions were made and yet the fit was good.

No  $F_4$  selections were made from family 15a-4, on which account grain color segregations were not obtained.

#### COLOR OF GLUMES

Each plant in each  $F_3$  progeny was classified for color of glumes. In family 15a-7 there were 38 families which bred true for bronze glumes and 50 which bred true for white glumes. The remaining 98 progenies segregated. This suggests a 1:2:1 ratio—that is, a one-factor difference. In family 15a-4 there were 16 true-breeding progenies for bronze glumes; 22 true for white; and 39 segregating for red and white. This also suggests a one-factor difference. The closeness of fits for this hypothesis are given in Tables 13 and 14.

TABLE 13.—*Closeness of fit of three groups of color of glumes compared to a 1:2:1 ratio*

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Group	C	O	C-O	(C-O) <sup>2</sup>	$\frac{(C-O)^2}{C}$
Homozygous bronze.....	46.5	38	8.5	72.25	1.5538
Heterozygous.....	93.0	98	-5.0	25.00	.2688
Homozygous white.....	46.5	50	-3.5	12.25	.2634
$\chi^2=2.0860 \quad P=0.3554$					

TABLE 14.—*Closeness of fit of three groups of color of glumes compared with a 1:2:1 ratio*

[Family 15a-4; grown in 1926 at Petersboro, Utah]

Group	C	O	C-O	(C-O) <sup>2</sup>	$\frac{(C-O)^2}{C}$
Homozygous bronze.....	19.25	16	3.25	10.5625	0.5487
Heterozygous.....	38.50	39	-.50	.2500	.0065
Homozygous white.....	19.25	22	-2.75	7.5625	.3929
$\chi^2=0.9481 \quad P=0.6189$					

In family 15a-7,  $P=0.36$ , and family 15a-4,  $P=0.62$ . It is highly probable, therefore, that the segregation is in a 1:2:1 ratio—that is, determined by a single-factor difference.

#### CORRELATION STUDIES

Simple, partial, and multiple correlations were made of the mean values of  $F_3$  progenies. As already mentioned, measurements or counts were taken on each  $F_3$  plant in each progeny, and from these the mean of the entire progeny was calculated for the 187  $F_3$  progenies in family 15a-7 and for the 77  $F_3$  progenies in family 15a-4.

All possible correlations were made between the five characters on which observations were taken. The combinations were as follows: (1) Head density × awn length; (2) head density × height of plant; (3) head density × number of spikelets; (4) head density × number

of culms; (5) awn length  $\times$  height of plant; (6) awn length  $\times$  number of spikelets; (7) awn length  $\times$  number of culms; (8) height of plant  $\times$  number of spikelets; (9) height of plant  $\times$  number of culms; and (10) number of spikelets  $\times$  number of culms.

In family 15a-4, part of the  $F_3$  progeny rows extended to a piece of shallow, stony, and less fertile land. The plants became noticeably shorter in a somewhat progressive but not very regular fashion in the region where the progeny rows with lax heads were growing. At first it was decided to abandon this family, but when the high correlation was obtained between spike density  $\times$  awn length in family 15a-7, these data were taken on family 15a-4 also. There was reason for believing from previous work that the two characters of spike density and awn length were stable enough not to be widely influenced by somewhat poor soil. Accordingly, the observations were made and the data calculated as for family 15a-7. As will be seen from Table 15, the only coefficients of correlation that differ materially from those obtained in family 15a-7 are the ones involving height of plant, and only two of these could be considered erratic—(1) spike density  $\times$  height of plant and (2) awn length  $\times$  height of plant.

TABLE 15.—Correlation coefficients ( $r$ ), correlation ratios ( $\eta$ ), their respective probable errors (P. E.), and Blakeman's test of linearity for various pairs of plant characters

[Means of  $F_3$  progeny rows, each of 35 to 40 plants, grown in 1926 at Petersboro, Utah]

Characters correlated	$r \pm$ P. E.	$r$ P. E.	$\eta \pm$ P. E.	$\eta$ P. E.	Blake- man's test
Spike density $\times$ awn length:					
15a-7.....	+0.6737 $\pm$ 0.027	25.0	0.7006 $\pm$ 0.025	28.0	1.9474
15a-4.....	+ .7619 $\pm$ .032	23.6	.8712 $\pm$ .026	33.5	1.9218
Spike density $\times$ height of plant:					
15a-7.....	+ .0696 $\pm$ .049	1.4	.3917 $\pm$ .042	9.4	3.908
15a-4.....	- .6001 $\pm$ .049	12.2	.7310 $\pm$ .036	20.4	2.7156
Spike density $\times$ number of spikelets:					
15a-7.....	- .3169 $\pm$ .044	7.1	.4676 $\pm$ .039	12.1	3.476
15a-4.....	- .2447 $\pm$ .072	3.4	.3406 $\pm$ .068	5.0	1.542
Spike density $\times$ number of culms:					
15a-7.....	+ .1235 $\pm$ .049	2.5	.3996 $\pm$ .041	9.7	3.852
15a-4.....	+ .0075 $\pm$ .077	.1	.3259 $\pm$ .069	4.7	2.1191
Awn length $\times$ height of plant:					
15a-7.....	+ .0985 $\pm$ .049	2.0	.2044 $\pm$ .047	4.3	1.8157
15a-4.....	- .4070 $\pm$ .064	6.3	.4980 $\pm$ .058	8.6	1.8661
Awn length $\times$ number of spikelets:					
15a-7.....	- .0629 $\pm$ .049	1.2	.2037 $\pm$ .047	4.3	1.9587
15a-4.....	- .016 $\pm$ .077	.2	.3185 $\pm$ .069	4.6	2.0692
Awn length $\times$ number of culms:					
15a-7.....	+ .1611 $\pm$ .048	3.4	.3586 $\pm$ .043	8.3	3.247
15a-4.....	+ .0377 $\pm$ .077	.5	.2982 $\pm$ .070	4.3	1.9239
Height of plant $\times$ number of spikelets:					
15a-7.....	+ .2273 $\pm$ .047	4.8	.4078 $\pm$ .041	9.9	3.422
15a-4.....	+ .4344 $\pm$ .063	7.0	.5024 $\pm$ .057	8.7	1.642
Height of plant $\times$ number of culms:					
15a-7.....	+ .3065 $\pm$ .045	6.9	.4401 $\pm$ .040	11.1	3.0599
15a-4.....	+ .1687 $\pm$ .075	2.3	.4671 $\pm$ .060	7.8	2.8333
Number of spikelets $\times$ number of culms:					
15a-7.....	+ .1950 $\pm$ .048	4.1	.2435 $\pm$ .047	5.2	1.474
15a-4.....	+ .2517 $\pm$ .072	3.5	.3195 $\pm$ .069	4.6	1.2796

The correlation coefficient ( $r$ ) and the correlation ratio ( $\eta$ ) were obtained for each of the 20 correlations. The respective probable errors and Blakeman's tests were calculated and tabulated as given in Table 15. Since spike density has been shown in previous papers and in this one to be a stably inherited character, its correlations with other characters are given first. Awn inheritance has been also shown to be stably inherited, and its correlations with the other characters are next in order. The correlations of other characters follow:

Spike density correlated with awn length gives in family 15a-7 a correlation coefficient ( $r$ ) of  $+0.6737 \pm 0.027$ ; in family 15a-4,  $r = +0.7619 \pm 0.032$ . In one case  $r$  is 25 times its probable error and in the other case 23.8 times. The correlation ratios ( $\eta$ ) are  $0.7006 \pm 0.025$  and  $0.8712 \pm 0.026$  and the ratios of  $\frac{\eta}{P. E.}$  are 28 and 33.5, respectively. Blakeman's tests for linearity in these two cases are 1.9474 and 1.9218, showing that the probability of the regressions being linear is about 1 in 4. These figures all denote clearly and definitely a high correlation between spike density and awn length, such as can be measured by  $r$ .

The nature of the correlation surfaces between spike density and awn length is shown in Tables 16 and 17. An examination of the frequency distributions in both tables will again emphasize the high degree of linear correlation and also the almost exact similarity between them.

TABLE 16.—Correlation between the characters spike density and awn length

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Spike-density classes (millimeters)	Awn-length classes (millimeters)														Total	
	51	54	57	60	63	66	69	72	75	78	81	84	87	90		93
18				1	3											4
21	1		2	4	6	7	3	4	2							29
24					3	3		5	1							12
27					1	2	4	2								9
30					2	6	6	3	4	2				1		24
33				1	1	4	8	7	11	7	4					43
36					1	1	5	6	4	4	1	1			1	24
39								2	1	1						4
42								1					1			2
45							1		2		3			1		7
48								2	2	2	3	1	2	2	1	15
51							1	2	2	2	1	2	1			11
54												1		1	1	3
Total	1	0	2	6	17	23	28	34	29	18	12	5	4	5	3	187

$$r = +0.674 \pm 0.027.$$

TABLE 17.—Correlation between the characters spike density and awn length

[Family 15a-4; means of  $F_2$  progenies grown in 1926 on rough, irregular, and relatively nonproductive soil, at Petersboro, Utah]

Spike-density classes (millimeters)	Awn-length classes (millimeters)														Total
	51	54	57	60	63	66	69	72	75	78	81	84	87		
21			1	1	1	1	1								4
24		1	1		1	2	6	3	2						16
27									1						1
30					3	5	1	2	1	1					13
33						3	3		1	2	3	1			13
36						1	1	2	2						6
39							1		1		1				3
42															0
45															0
48									1	1	2	3	1		8
51							1		2	2	1	1		1	8
54										1	1	1	1		4
57														1	1
Total	1	1	2	6	16	11	6	8	7	7	5	4	3		77

$$r = +0.762 \pm 0.032.$$

Aside from the high correlation in each of the two families, the almost exact correspondence of the two  $r$ 's, the two  $\eta$ 's, and the two Blakeman's tests is further evidence of a high order that there is a close correlation such as  $r$  can measure between spike density and awn length.

This correlation was also studied in another way. There was a series of  $F_3$  progenies which were segregating for spike density. Since the awns on individual plants were measured it was possible to obtain the correlation between spike density  $\times$  awn length within each progeny. Altogether, 13 such progenies chosen at random were correlated, with the results given in Table 18.

TABLE 18.—Correlation coefficients ( $r$ ) between spike density and awn length in 13

$F_3$  progenies, together with the probable errors (P. E.) and the ratio  $\frac{r}{P. E.}$   
[Individual  $F_3$  plants; grown in 1926 at Petersboro, Utah]

$F_3$ progeny	Correlation coefficient $r \pm P. E.$	$\frac{r}{P. E.}$
15a-7-56.....	+0.3808 $\pm$ 0.091	4.18
15a-7-61.....	+ .5735 $\pm$ .076	7.55
15a-7-69.....	+ .4396 $\pm$ .089	4.94
15a-7-75.....	+ .3325 $\pm$ .104	3.20
15a-7-82.....	+ .6726 $\pm$ .061	11.03
15a-7-87.....	+ .2291 $\pm$ .102	2.25
15a-7-93.....	+ .5351 $\pm$ .076	7.04
15a-7-105.....	+ .5803 $\pm$ .071	8.17
15a-7-116.....	+ .2986 $\pm$ .098	3.04
15a-7-124.....	+ .3695 $\pm$ .093	3.97
15a-7-132.....	+ .5882 $\pm$ .076	7.74
15a-7-140.....	+ .3701 $\pm$ .092	4.02
15a-7-145.....	+ .4490 $\pm$ .098	4.58

The  $r$ 's vary from  $+0.2291 \pm 0.102$  to  $+0.6726 \pm 0.061$ , and the ratio of  $\frac{r}{P. E.}$  varies from 2.24 to 10.94. In only one case is  $r$  less than three times its probable error. In 5 out of the 13 cases, the  $r$ 's are seven to eleven times their respective probable errors. In the other seven cases the ratio of  $\frac{r}{P. E.}$  is between 3 and 5. All of these  $r$ 's are plus, and all of them except one show odds of 25, or more, to 1 of being significant. In 9 of the 13 cases the odds are from 150 to infinity to 1 that the correlations are significant; 5 of the 9 have odds above 450,000 to 1.

The fact that all the correlation coefficients ( $r$ ) are plus means that the longer the heads the longer the awns.

This evidence bears out strikingly the conclusion previously arrived at, largely by use of correlation ratios ( $\eta$ ), that "between the two stable and definitely inherited characters of spike density and awn classes it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters."<sup>6</sup>

Incidentally, also, here is evidence of the considerable importance of biometrical methods in analyzing genetic data. In the previous study the awns were grouped into four classes by inspection and the spike density was measured. The constant  $r$  is unadapted for correlation studies where one of the characters is grouped by categories

<sup>6</sup> STEWART, G. 1926. Op. cit.



rather than by actual discrete classes. The correlation ratio  $\eta$ , however, measures such correlations rather satisfactorily, and gave with sufficient clearness the indication that it was worth while to study the correlation between spike density and awn length with the care and the detail here reported.

In the correlations between spike density and height of plant,  $r$  is  $+0.0696 \pm 0.049$  in family 15a-7;  $\eta$  is  $0.3917 \pm 0.042$ . In the other family,  $r$  is  $-0.6001 \pm 0.049$  and  $\eta$  is  $0.7310 \pm 0.036$ . In view of the contradiction between the two  $r$ 's and in view of the fact that height of plant was markedly decreased by the poor and irregular soil on which some of the progenies of 15a-4 were grown, it does not seem wise to attach great importance to the  $r$ 's themselves. On the other hand, both of the  $\eta$ 's are significant in the light of their probable errors, being 9.4 and 20.4 times their respective probable errors. Of the two Blakeman's tests one is just less than 4 and the other just less than 3. Since in the previous study the  $\eta$ 's were also large enough to suggest a correlation, and since all of the Blakeman's tests were likewise rather large, there seems to be at least a moderate suggestion of the presence of a correlation of some sort between spike density and height of plant. Since the  $r$ 's were all very small in the previous study, and since in this study one is small and the other is known to be unreliable, there seems to be very little correlation such as  $r$  can measure. Repetition of the cross, with the  $F_3$  progenies grown in careful replications and checked by being grown adjacent to the parental strains, would be necessary in order to measure this correlation. Sufficient refinement in the planting plan to permit an approximation of the variability due to soil heterogeneity would also be necessary.

When spike density is correlated with the number of spikelets to the spike, the two  $r$ 's are  $-0.3169 \pm 0.044$  and  $-0.2447 \pm 0.072$ , and the two  $\eta$ 's are  $0.4676 \pm 0.039$  and  $0.3406 \pm 0.068$ . The  $r$ 's are 7.1 and 3.4 times their probable errors, and the  $\eta$ 's 12.1 and 5 times their respective probable errors. The figures for the two Blakeman's tests are 3.4 and 1.5 for the two cases. There seems to be a suggestion of correlation between spike density and the number of spikelets, as indicated both by  $r$  and by  $\eta$ .

These two plant characters were not correlated in the previous study. Careful planning of an experiment should permit the measurement of this correlation.

The two  $r$ 's between spike density  $\times$  number of culms to the plant are so small that they should not be given much importance. The  $\eta$ 's, however, are proportionately larger ( $0.3996 \pm 0.041$  and  $0.3259 \pm 0.069$ ), which are 9.7 and 4.7 times their probable errors. No earlier correlations were made between these two characters.

Awn length correlated with height of plant gave one very small  $r$  and one fairly large one which is thought in this case (family 15a-4) to be the result of the poor development in height due to poor soil. It is probable that any study involving height of plant in family 15a-4 is unreliable. The correlations obtained, therefore, should not be considered significant, unless the  $\eta$  for family 15a-7 ( $\eta = 0.2044 \pm 0.047$ ), which is reliable, should be so considered. This constant is 4.3 times its probable error. There was no other study to substantiate this suggestion of a correlation, as even the  $\eta$ 's in the previous study for awn classes  $\times$  plant height were small. Studies for awn

classes have only a suggestive bearing on measured awn-length relationships.

The correlation coefficients ( $r$ ) for awn length  $\times$  number of spikelets are negligible, being  $-0.0629 \pm 0.049$  and  $-0.016 \pm 0.077$ . The  $\eta$ 's are  $0.2037 \pm 0.047$  and  $0.3185 \pm 0.069$ , both a little more than four times their probable errors. Since there were no additional studies to corroborate these correlations, no definite conclusions can be drawn without further study.

The two  $r$ 's for awn length  $\times$  number of culms are small both in absolute size and in relation to their probable errors. The  $\eta$ 's are considerably larger ( $0.3586 \pm 0.043$  and  $0.2982 \pm 0.070$ ), one being 8.3 and the other 4.3 times their probable errors. These four rather uniform results might be considered at least to suggest an important relationship. This is, however, decreased by the failure to establish definite evidence of inherited differences between the two parental strains in the number of culms to the plant.

Height of plant  $\times$  number of spikelets and height of plants  $\times$  number of culms both yielded  $r$ 's and  $\eta$ 's which on the surface would seem to be suggestive. These constants are all fairly large and are from 4.8 to 11.1 times their probable errors, except in one case which is 2.3 times its probable error. The fact that there may be a general tendency for plant height and number of culms to respond in the same way to environmental conditions weakens the value of these constants. It is elementary information that both of these two characters are extremely variable in the small cereals. They might even be physiologically correlated—that is, due to the same genetic factor. The case is not so clear in the correlation of height of plant  $\times$  number of spikelets, for the number of spikelets is not nearly so variable as the height or the stooling characters. Carefully replicated plantings of good genetic material might reveal correlations between height of plant and number of spikelets. The  $r$ 's in this case are  $+0.2273 \pm 0.047$  and  $+0.4344 \pm 0.063$ , and the  $\eta$ 's are  $0.4078 \pm 0.041$  and  $0.5025 \pm 0.057$ . The ratios of  $\frac{r}{P. E.}$  are 4.8 and 7, while the ratios of  $\frac{\eta}{P. E.}$  are 9.9 and 8.7. These probably suggest real correlations, but would require much good statistical work to establish.

The study of number of spikelets  $\times$  number of culms gave  $r$ 's of  $+0.1950 \pm 0.048$  and  $+0.2517 \pm 0.072$ , which are 4.1 and 3.5 times the probable errors. The  $\eta$ 's are of about the same order ( $0.2435 \pm 0.047$  and  $0.3195 \pm 0.069$ ), being 5.2 and 4.6 times their respective probable errors. Both Blakeman's tests are small and indicate that whatsoever correlation exists between these two plant characters is linear.

#### PARTIAL AND MULTIPLE CORRELATIONS

In order to learn more nearly the true relationship between the various plant characters, partial correlations were computed. Partial correlation gives the degree of relationship between any two characters when the other variables are held constant. In these calculations the plant characters are numbered as follows:

(1) Spike density, (2) awn length, (3) height of plant, (4) number of spikelets per spike, and (5) number of culms per plant.

Partial correlations between spike density and awn length is designated as  $r_{12.345}$ , which means that the true relationship between characters No. 1 (spike density) and No. 2 (awn length) is obtained by holding constant the other three variables, 3, 4, and 5. The designation  $r_{24.135}$  means that the true relationship between characters No. 2 (awn length) and No. 4 (number of spikelets) is found by holding constant characters 1, 3, and 5. Ten combinations of partial correlations are possible. These are shown in Table 19 beside the product-moment correlation coefficients ( $r$ ) already given. Since family 15a-4 had been disturbed environmentally, partial correlations were calculated only for family 15a-7.

TABLE 19.—*Simple product-moment correlation coefficients ( $r$ ) and comparative partial correlations between five plant characters in the wheat cross Kanred × Sevier*

[Family 15a-7; on means of  $F_3$  progenies, grown in 1926 at Petersboro, Utah]

Product-moment correlations		Partial correlations	
Characters correlated	Correlation coefficients	Partial correlations	Correlation coefficients
Spike density × awn length.....	+0.6737 ± 0.027	$r_{12.345}$	+0.6500 ± 0.028
Spike density × plant height.....	+ .0696 ± .049	$r_{13.245}$	+ .0957 ± .049
Spike density × number of spikelets.....	- .3169 ± .044	$r_{14.235}$	- .3796 ± .042
Spike density × number of culms.....	+ .1238 ± .049	$r_{15.234}$	+ .0833 ± .049
Awn length × plant height.....	+ .0985 ± .049	$r_{23.145}$	+ .0045 ± .049
Awn length × number of spikelets.....	- .0629 ± .049	$r_{24.135}$	+ .1737 ± .048
Awn length × number of culms.....	+ .1611 ± .048	$r_{25.134}$	+ .0625 ± .049
Plant height × number of spikelets.....	+ .2273 ± .047	$r_{34.125}$	+ .1997 ± .047
Plant height × number of culms.....	+ .3065 ± .045	$r_{35.124}$	+ .2488 ± .046
Number of spikelets × number of culms.....	+ .1950 ± .048	$r_{45.123}$	+ .1643 ± .048

The partial correlations and the simple product-moment correlations show important size in the same places. The simple product-moment correlation between awn length and number of spikelets was  $-0.0629 \pm 0.049$ , whereas the partial correlations increased this to  $+0.1737 \pm 0.048$ , indicating that the true correlation was somewhat higher than was shown by the product-moment coefficient ( $r$ ). There was no other material increase or decrease in size in any of the partial correlation coefficients as compared with the same respective product-moment coefficients.

Especially worthy of notice is the partial correlation for spike density × awn length when plant height, number of spikelets, and number of culms are held constant ( $r_{12.345}$ ) =  $+0.6500 \pm 0.028$ ). This also corroborates the previous conclusion that there is a real correlation between spike density and awn length. The partial correlation of  $r_{14.235}$  =  $-0.3796 \pm 0.042$  gives a fairly strong correlation between spike density and number of spikelets when awn length, plant height, and number of culms are held constant.

A partial correlation coefficient of  $+0.1997 \pm 0.047$  for plant height × number of spikelets and of  $+0.1643 \pm 0.048$  for number of spikelets × number of culms suggest some, though probably not high, correlations. The tendency of plant height and number of culms to keep together is again apparent, but perhaps has also again to be discounted due to the strong positive influence of favorable soil conditions on both plant characters.

Multiple correlations were calculated for each of the five plant characters in relation to all of the other four characters—that is, the total effect of the other four characters on spike density is indicated by  $R_{1.2345} = 0.7333$ . The actual value of this figure can be obtained in percentage from the equation  $v$  (per cent) =  $100(1 - \sqrt{1 - r_2^2})$ . When this calculation is made, it is found that 32 is the solution value obtained. Only 32 per cent, therefore, of the total variation in spike density is accounted for by the variation in the other four characters. This leaves 68 per cent of the variation to be explained by causes other than the ones here studied. Soil heterogeneity could not be determined from the data taken in this study. General observation of the soil used, in comparison with soils on which heterogeneity had been measured, would indicate it to be rather high in this experiment. The soil of the experimental field used was somewhat less uniform than another on which 15 per cent variability has been found. A brief summary of the multiple correlations for each character in relation to the other four is given in Table 20.

TABLE 20.—Multiple correlations showing the total effect of the variability caused by the other four plant characters on the respective one indicated, in the wheat cross *Kanred* × *Sevier*

[Family 15a-7; means of  $F_2$  progenies, grown in 1926 at Petersboro, Utah]

Plant character	Multiple correlation	Correlation coefficient	Percentage of total variability accounted for
Spike density.....	$R_{1.2345}$	0.7333	32.0
Awn length.....	$R_{2.1345}$	.6591	25.4
Plant height.....	$R_{3.1245}$	.3784	7.5
Number of spikelets.....	$R_{4.1235}$	.4700	11.8
Number of culms.....	$R_{5.1234}$	.3647	6.9

As already noted, 32 per cent of the variation in spike density is accounted for in the variation of the other four characters studied. In the case of awn length 25.4 per cent of the variation is cared for, leaving 74.6 per cent due to unmeasured causes. In the other three cases, only 7.5, 11.8, and 6.9 per cent of the variation are accounted for by causes here studied.

Apparently only spike density and awn length account for any appreciable amount of the variation in the other one of this pair of characters. Even though far from complete in their influence, correlations which account for 32 and 25 per cent of the total variation must be ranked as important.

### SUMMARY

This study is the outgrowth of a previous paper<sup>7</sup> in which the presence of a correlation was indicated between awn classes and spike density. In the previous study, a cross of *Sevier* × *Federation*, plants were grouped into four classes according to their expression of awns. There was a wide range in spike density in the  $F_2$  progenies.

<sup>7</sup> STEWART, G. 1926. Op. cit.

Correlation ratios indicated considerable correlation between the measured spike densities and the awn classes.

The material used in the present study, a cross of Kanred  $\times$  Sevier, was at that time being grown as part of an economic plant-breeding project. The fact that both parents were fully awned would have precluded any study of the awn character had not the hybrid progenies proved to be most desirable genetic material with which to study the correlation between spike density and awn length. There was a wide transgressive segregation in spike density and this seemed to be accompanied by differential length of awns. Accordingly,  $F_3$  progenies seeded as a means of obtaining pure lines for rod-row yield tests were studied genetically.

The contrasting characteristics in the parental strains were as follows:

<i>Kanred</i>	<i>Sevier</i>
Spike density lax.	Spike density intermediate.
Fully awned.	Fully awned (awns shorter than those of Kanred).
Culms intermediate.	Culms long.
Kernels red.	Kernels white.
Glumes white.	Glumes bronze.

No exact data were taken in  $F_1$ , and in  $F_2$  only color of grain was observed with care. Since all  $F_2$  plants had been continued in  $F_3$  progenies of 30 to 40 spaced plants each, these were studied, and the breeding behavior of each progeny taken as the true genotype of the  $F_2$  plant from which it was descended. Each figure in the tables reported represents, therefore, a result obtained from studying each of the 30 or 40 plants in the  $F_3$  progeny. Interspersed among the hybrids as they grew in the field were 8 rows of Sevier and 10 rows of Kanred. These were studied in the same fashion as the hybrids, each row being regarded as a unit. Three or four times as many parent rows would have been desirable. Of the hybrid progenies there were 187 in family 15a-7 and 77 in family 15a-4.

Spike density measurements of 10 central spikelet internodes were taken in millimeters on one leading spike of each plant in each progeny. The length of awns on the same spike was also measured in millimeters. The plant height was taken in centimeters to the base of the spike on the longest culm. After counting the number of culms on each plant and the number of spikelets on the spike measured for density, the worker who took the data classified each plant as to grain color and glume color.

#### INHERITANCE OF CHARACTERS

The  $F_2$  genotypes as classified by their  $F_3$  breeding behavior gave a close approximation of a 1:2:1 ratio for dense, heterozygous, and lax spikes. The dense-spiked and the lax-spiked progenies were true breeding. This was indicated by their coefficients of variability, which were approximately 10 per cent. That the progenies of intermediate spike density all segregated was indicated by coefficients of variability of 25 to 40 per cent. The numbers of homozygous dense, heterozygous, and homozygous lax progenies were, respectively, 45, 102, and 39 in one family, and 20, 36, and 21 in a second family, both of which are excellent fits for a 1:2:1 ratio.

In each of the three major groups of hybrids there was, however, a series of differences between various progenies. Some true-breeding dense progenies had considerably more dense spikes than did others. This was equally true of the heterozygous and of the lax progenies as well. The coefficients of variability for the means of the spike density of the hybrid progenies were about twice as great as for the parental rows. This is good evidence of the presence of minor factors in spike density inheritance. These minor factors were of such a nature as to prevent the definite recovery of the spike density of Sevier in a single true-breeding  $F_3$  progeny. Only one of the 263 progenies studied even approached the Sevier parent in head density. There was a difference between the mean of the progeny and the mean of the most dense row of Sevier of about 1.5 times the probable error.

Kanred was distinctly shorter than Sevier. Some progenies were obtained, the mean height of which was somewhat greater than that of either parent, though some Kanred rows were shorter than any of the hybrid progenies. The total range in height of the progenies was about equal to the range covered by the two parents. Though segregation in height was clearly manifest, no ratios were found which indicated the nature of the segregation. No definite conclusions were reached as to the inheritance of the number of spikelets or the number of culms.

The inheritance of grain color gave a close fit between the expected and the observed on the basis of a three-factor difference. In  $F_3$  and  $F_4$ , true-breeding red-grained progenies and true-breeding white-grained progenies were obtained, as well as others segregating 63:1, 15:1, and 3:1 for red and white grain.

A ratio of 3 bronze-chaffed progenies to 1 white-chaffed was obtained

#### CORRELATION STUDIES

Simple, partial, and multiple correlations were calculated in all possible combinations between (1) spike density, (2) awn length, (3) height of plant, (4) number of spikelets, and (5) number of culms.

Spike density and awn length gave high correlations. In two families  $r$  was  $+0.674 \pm 0.027$  and  $+0.762 \pm 0.32$ , which are 25 and 23.6 times their probable errors. In addition, 13 progenies segregating for spike density gave  $r$ 's which varied from  $+0.229 \pm 0.102$  to  $+0.673 \pm 0.061$ . In 12 of these 13 correlations,  $r$  is more than 3 times its probable error and in 5 cases from 7 to 11 times the probable error. Every  $r$  between spike density and awn length was plus, indicating that the longer spikes had longer awns. The partial correlation was also high, being  $+0.6500 \pm 0.028$  when height of plant, number of spikelets, and number of culms were held constant. In the multiple correlations for spike density and awn length,  $R$  equaled 0.733 and 0.659, respectively.

There seems no doubt, therefore, that there is linkage between spike density and awn length. There is also a suggestion of correlation between spike density and number of spikelets and between height of plant and number of spikelets.

# A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE<sup>1</sup>

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## INTRODUCTION

In studying the large brown-patch disease of turf, described by Piper and Coe (6)<sup>2</sup> as due to *Rhizoctonia solani*, it was found that the causal organism varied in culture in several respects from some stock cultures of *R. solani*<sup>3</sup> obtained from potato tubers. There was found a marked difference in their pathogenicity on grass, and it seemed possible that they might be distinguished by certain variations in growth characteristics on artificial media. The investigations here reported were undertaken in an attempt to determine to what extent such characteristics would prove reliable in distinguishing the *Rhizoctonia* on grass from the potato organism, either as a distinct species or as a strain of *R. solani*. Several cultures were therefore obtained from different sources to compare with the grass fungus under various cultural conditions.

## SOURCE OF STRAINS

Two isolations of the *Rhizoctonia* producing brown patch of grass were used in these comparisons. One of these (1) was obtained from diseased bent-grass leaves growing in the greenhouse at Madison, Wis., in the spring of 1924, and the other (2) was isolated in June of the same year from grass injured by brown patch in the turf garden on the Arlington Experiment Farm, Rosslyn, Va. Since the turf growing in the greenhouse at Madison was originally sent from the Arlington turf garden, these two cultures were probably of the same origin.

Cultures 3 and 4 were obtained from B. L. Richards, isolated from potato and identified by him as *Corticium vagum* B. and C. These two represent his cultures Nos. 35 and 131, respectively. Cultures 5 and 6 were isolated by Freeman Weiss in Washington from diseased potato tubers during the summer of 1924. Culture 7 was isolated from a sclerotium on a potato tuber at Madison in January, 1925. Culture 8 was from a stock culture in the laboratory of plant pathology at Madison, Wis. This represents the culture known as Rosenbaum's strain R5, isolated in 1916 in Maine from a diseased potato stem. Culture 9 was isolated from diseased peas in the spring of 1924 by F. R. Jones in Wisconsin.

<sup>1</sup> Received for publication Dec. 1, 1927; issued July, 1928. Work done in the department of plant pathology, University of Wisconsin, in cooperation with the United States Golf Association Green Section.

<sup>2</sup> Reference is made by number (italics) to "Literature cited," p. 903.

<sup>3</sup> No attempt will be made to make detailed references to the extensive literature dealing with this fungus. The writers are aware of the divergence of opinion as to what constitutes a "species" or "strain" of *Rhizoctonia*, as expressed in such publications as those by Duggar (2), Peltier (3), Matz (4), Matsumoto (5), Britton-Jones (7), and Thomas (7).

## METHODS

Differences between the fungi isolated from grass and those from potato proved to be practically the same on several culture media, so it was decided to confine the study chiefly to a single medium. Potato-dextrose agar was chosen for this purpose, since it proved to be favorable for all the cultures, besides having several other desirable features. This was prepared in the usual manner, a decoction of 200 gm. of potato per liter of the medium with 2 per cent dextrose and  $1\frac{1}{2}$  per cent agar being used. In pouring plates sufficient agar was used to make a layer about 5 mm. thick. This provided a sufficient reserve of moisture to take care of any small loss due to evaporation in cultures grown for several days. Tests showed that there were decided differences in initial growth, depending on whether transfers were made from old or from young cultures. In some cases inoculations made with the tips of growing mycelium had entirely crossed the plate before similar inoculations made from old cultures had started to develop. Therefore all further inoculations were made by transferring pieces of agar containing tips of growing mycelium taken from the borders of young cultures.

## INFLUENCE OF TEMPERATURE ON RATE OF GROWTH

The influence of temperature on the rate of growth was studied in incubators maintained at 10°, 15°, 20°, 25°, 30°, and 35° C. In the preliminary tests, when Petri-dish cultures were piled together in one of the temperature chambers, there was frequently a noticeable difference in growth in the dishes in the center of the pile compared with those at the top or bottom. These differences were most marked in chambers where the temperatures were well above or below that of the laboratory and apparently were dependent on the time required for the plates to reach a temperature equilibrium. Therefore, in subsequent work the medium was poured and the dishes were kept in their respective incubators for several hours to bring them to the desired temperature before inoculating. Transfers were made and later observations were recorded as rapidly as possible, to avoid any serious temperature change while the material was out of the incubator.

As the work progressed it was found that at a given temperature a more vigorous growth frequently occurred if the transfer was made from a culture grown at this same temperature than if the transfer was made from a culture grown at some other temperature. Thus, if two cultures were kept at 15°, one inoculated from a culture grown at 15° and the other from one at 25°, the transfer from the 15° plate would make a much more rapid growth. This behavior was more marked in the case of certain cultures than in others. To avoid this difference, therefore, inoculations for any temperature were made from a plate previously kept at that same temperature.

When transfers were made from the same plate and even from corresponding segments of mycelium there was some noticeable difference in the rapidity with which the new cultures started growth, although when once established they grew at very nearly the same rate. To avoid this initial difference, measurements were made about 24 hours after transferring, at which time new growth had



started in most cases. Readings were again made when the mycelium had reached the edge of the plate at the optimum temperature. From these measurements the rate of radial growth per hour during this interval was calculated. Figure 1 shows the average growth for a typical temperature series, based on these calculations.

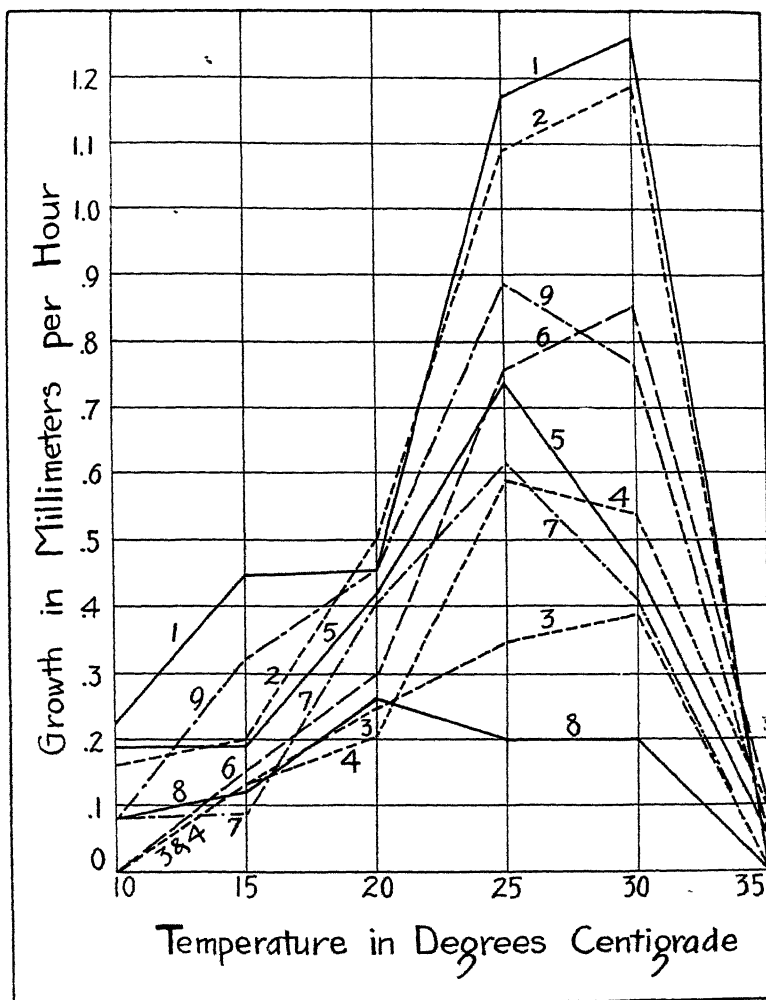


FIG. 1.—Effect of temperature on rate of growth of nine cultures of *Rhizoctonia solani* on potato-dextrose agar

It will be seen that in general the optimum temperature for growth was 25° or 30°. The two grass strains (1 and 2) showed a similar response to temperature, as might be expected, since their origin was similar. At 25° and 30° these cultures were much more vigorous than any of the potato strains. The difference between them and certain potato strains, however, was not so great as the difference existing between various strains isolated from potato tubers. In

comparing the curves it is seen that the cultures which had been kept on artificial media for the longest periods, namely, 3, 4, and 8, grew less vigorously than some of the more recent isolations. This might indicate a decrease in vigor due to continued artificial culture. However, it will be noted that the fungus representing the shortest period in culture, No. 7, was also one of the less vigorous group.

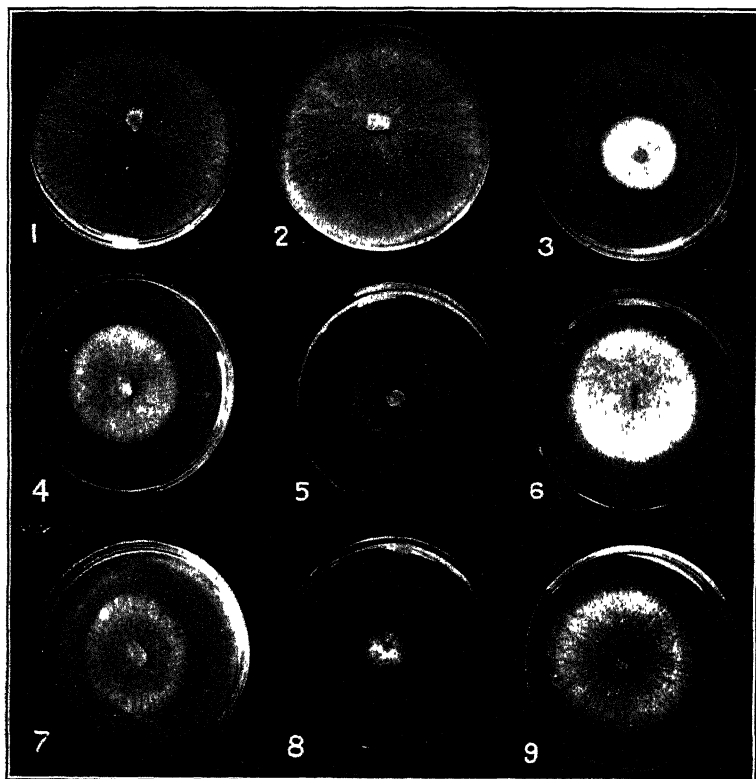


FIG. 2.—Nine cultures of *Rhizoctonia solani* grown on potato-dextrose agar at 25° C. Cultures 1 and 2 were isolated from grass, and the others were obtained from potatoes from different localities. The two from grass could readily be distinguished from the others at this temperature, but, owing to the wide variation shown in the potato strains, such differences would probably be of little value in distinguishing strains if a large collection were studied.

#### INFLUENCE OF TEMPERATURE ON CHARACTER OF GROWTH

When the cultures were several days old there were striking differences in the appearance of the colonies at different temperatures. An attempt was made to distinguish strains based on the character of growth. For this purpose such characters as color of mycelium or sclerotia, discoloration of medium, amount and type of aerial growth, shape and size of sclerotia, and various other such characters were used.

From these observations it was evident that certain cultures could be distinguished readily from others. Figure 2 shows a group of

typical colonies of the same age grown at 25° C. This illustrates the close similarity between the two grass cultures, 1 and 2. These are readily distinguished from such potato cultures as 3 and 6. The differences in these cases, however, are no more marked than are the differences between such strains as 5 and 6, which were both isolated from potato tubers at about the same time. These macroscopic differences were furthermore complicated by changes at other temperatures; for instance, cultures 5 and 7 had very much the same appearance at 25°, but at some of the other temperatures they could be readily distinguished by their growth characters. In some cases there was a striking difference in type of growth with a change of only a few degrees of temperature. Figure 3 shows such a difference in the case of culture 6, which has a decidedly different type of growth

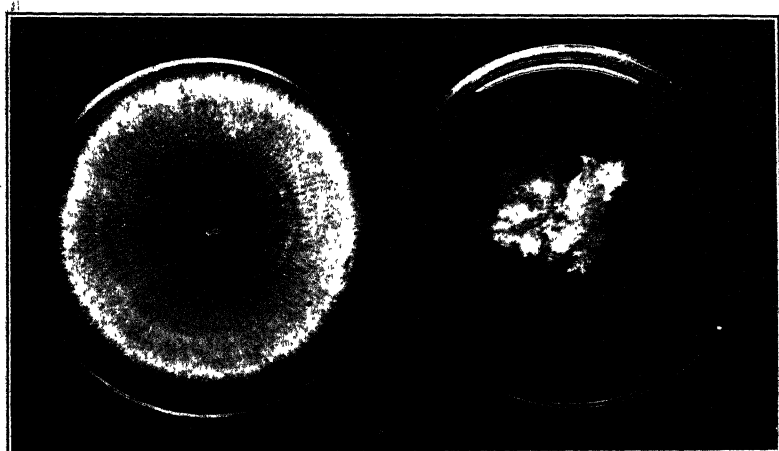


FIG. 3.—Strain No. 6, grown at 33° (left) and 35° C. (right) on potato-dextrose agar. The 33° culture was 3 days old, whereas the 35° culture had been growing 7 days

at 33° and at 35°. In other cases, as in the two grass cultures, the growth characters were sufficiently constant throughout the temperature range to enable one to distinguish them from the other cultures.

The extent to which media are discolored is a characteristic sometimes used in distinguishing strains of *Rhizoctonia*. Nos. 1 and 2, from grass, did not noticeably darken the agar at any temperature. No. 8 (Rosenbaum's R5 strain) darkened agar very markedly at 20°, 25°, and 30°, but caused no discoloration at 15° C. Cultures 6 and 9 did not blacken the agar at any temperature except 35°, but there the discoloration was very distinct.

Cultures 1, 6, and 7 were grown for three months in flasks containing a mixture of corn meal and sand. These were kept at approximately 15°, 25°, and 30° C. Cultures 1 and 7 produced large compact sclerotia at 15° and much smaller and less compact masses at 30°. Culture 6, on the other hand, did not produce any at 15°, but produced many small sclerotia at 30° C. Observations on color as well as on type of sclerotia failed to reveal any reliable means for distinguishing the grass strain from the fungus found on potato.

## INFLUENCE OF TEMPERATURE ON DIAMETERS OF HYPHAE

To determine the extent to which the diameters of the hyphae might be used as a guide to distinguish strains, a series of measurements was made on three cultures growing at 15°, 20°, 25°, and 30° C. When these were 2 days old, cover glasses were placed over the outer edges of the colonies and measurements were made near the tips of the new hyphae, a point just back of the origin of the most recent lateral branch being selected. One hundred such measurements were made on duplicate plates for each temperature. The averages of

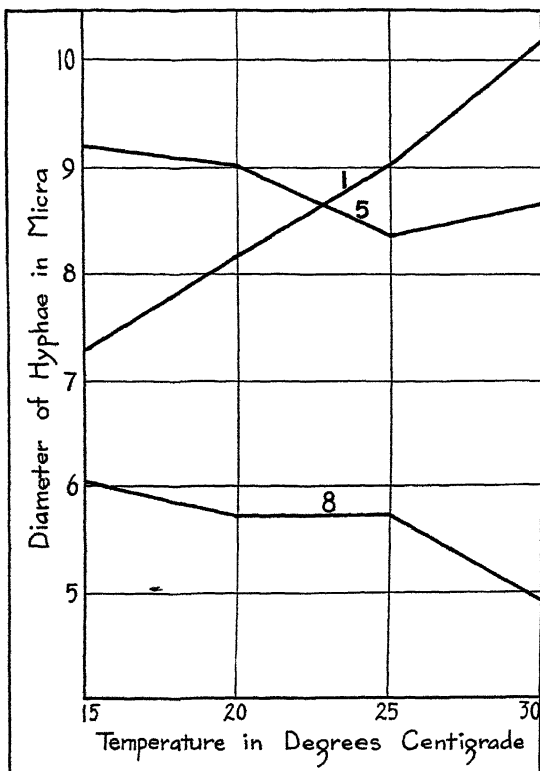


FIG. 4.—Variations in diameters of terminal hyphae in three strains of *Rhizoctonia solani* grown on potato-dextrose agar at different temperatures

These measurements are given in Figure 4, which shows the variation found in the diameters of the hyphae grown at different temperatures. It will be noted that the grass culture tended to increase rapidly in size with increase in temperature. This corresponds fairly closely with the increased radial growth illustrated in Figure 1. On the other hand, the other two from potatoes in general showed a tendency toward a decrease in diameter of hyphae with increase in temperature. These differences, however, are comparatively slight. A comparison of the diameter of growth at 15° and 30°, especially in the cases of 1 and 5, indicates how confusing it may be to use diameters of hyphae as a characteristic for distinguishing strains if the temperature factor is not considered.

## INFLUENCE OF ACIDITY OF MEDIUM ON GROWTH

*Rhizoctonia* is apparently able to grow over a wide range in degree of acidity or alkalinity. Variations in acidity of the culture medium, as expressed on the  $P_H$  basis, produced marked variations in the growth character of different cultures. Thus, color variations, from hyaline to dark brown, were observed in mycelium, the darker colors occurring usually near the neutral point. The type and color of sclerotia in each culture also showed marked variations due to degree of acidity. These differences, however, were not confined to, nor

sufficiently correlated with, any one culture or group of cultures in a way to justify their use as a key to differentiating strains.

#### SUMMARY AND CONCLUSION

In comparing on media cultures of *Rhizoctonia* isolated from diseased grass, numerous minor differences were found between them and cultures of this fungus isolated from potato. An attempt was made to determine to what extent these differences could be used to define the relationship of the *Rhizoctonia* found on grass to that obtained from potato or other hosts. Cultures of *Rhizoctonia solani* from different sources were therefore compared on several media under various conditions of temperature and acidity.

Using as a criterion various macroscopic and microscopic characteristics of growth such as have been used at times to define strains or even "species" of *Rhizoctonia*, it was found that temperature and acidity of the medium frequently so modified these characters that they appeared unreliable as dividing lines between species or even "strains" unless much detailed definition of certain environmental factors be included. Two cultures isolated from grass under most of the conditions studied could be distinguished from all other cultures of *Rhizoctonia* under observation. However, since each culture isolated from potato gave a somewhat different response, it is quite probable that if a sufficient number had been included there would have been found gradations covering the whole range between any of the extremes observed in this work.

The most outstanding distinction found in the grass fungus was its more rapid growth, especially at 25° and 30° C. This, however, is more likely to be a coincidence than an actual difference existing between all *Rhizoctonia* on grass and on potato.

From this study it is therefore concluded that the morphological and physiological differences observed between the *Rhizoctonia* causing large brown-patch disease on grass and stock cultures of *Rhizoctonia solani* are not sufficiently fixed and definite to serve as a reliable means for separating it from the variable fungus found on potato and other plants which is at present included in the species *R. solani*.

#### LITERATURE CITED

- (1) BRITON-JONES, H. R.  
1924. STRAINS OF RHIZOCTONIA SOLANI KÜHN (CORTICIUM VAGUM BERK. & CURT.) Brit. Mycol. Soc. Trans. 9: 200-210.
- (2) DUGGAR, B. M.  
1915. RHIZOCTONIA CROCORUM (PERS.) DC. AND R. SOLANI KÜHN (CORTICIUM VAGUM B. & C.) WITH NOTES ON OTHER SPECIES. Ann. Missouri Bot. Gard. 2: 403-458, illus.
- (3) MATSUMOTO, T.  
1921. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA SOLANI KÜHN. Ann. Missouri Bot. Gard. 8: 1-62, illus.
- (4) MATZ, J.  
1921. THE RHIZOCTONIAS OF PORTO RICO. Jour. Dept. Agr. Porto Rico, v. 5, no. 1, 30 p., illus.
- (5) PELTIER, G. L.  
1916. PARASITIC RHIZOCTONIAS IN AMERICA. Ill. Agr. Expt. Sta. Bul. 189, p. 281-390, illus.
- (6) PIPER, C. V., and COE, H. S.  
1919. RHIZOCTONIA IN LAWNS AND PASTURES. Phytopathology 9: 89-92, illus.
- (7) THOMAS, K. S.  
1925. ONDERZOEKINGEN OVER RHIZOCTONIA. 97 p., illus. Utrecht.



# DIFFERENCES IN RESISTANCE TO BACTERIAL WILT IN INBRED STRAINS AND CROSSES OF DENT CORN<sup>1</sup>

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## INTRODUCTION

Bacterial wilt or Stewart's disease of sweet corn, caused by *Aplanobacter stewarti* (E. F. S.) McC., has been known for more than 30 years (11).<sup>2</sup> Further studies have added to the knowledge of its host range, geographical distribution, methods of dissemination, and economic importance (1, 3, 4, 5, 8, 9, 10). Many varieties of sweet corn have been tested to determine their relative susceptibility to this disease (2, 3), and the value of seed treatment for its control has been investigated (3, 7); yet little has been accomplished in reducing the annual losses. In fact, the losses have tended to increase because of the commercial practice of growing early-maturing, highly susceptible sweet-corn varieties, such as Golden Bantam, in States where the disease is prevalent. The short-season sweet-corn varieties, as a group, are susceptible to bacterial wilt. It is probable, therefore, that if late planting becomes necessary in corn-borer control the wilt problem will be accentuated.

It is known that the prevalence of bacterial wilt of sweet corn decreases from south to north in the United States. The disease is seldom found very far north of the Corn Belt proper. From the standpoint of resistance and susceptibility, the late-maturing varieties of sweet corn are comparatively resistant and the early maturing varieties are susceptible. Dent corn has been found to be more resistant than sweet or flint corn (5). If a few exceptional years are left out of consideration, it may be said that losses from this disease in late varieties of sweet corn and in dent corn are of minor importance.

From the above-stated facts the control of bacterial wilt would seem to be simple; that is, (1) in the Corn Belt, where the disease occurs, grow the late-maturing varieties, which are resistant; and (2) north of where the disease occurs, grow the short-season, susceptible varieties, if desired. Although corn-canning companies could give greater consideration to these facts than they do at present, the market gardeners and home gardeners are limited to their localities and prefer to grow the popular varieties whenever they can. The high-quality varieties for table use on the ear, in general, are early maturing. At present no such high-quality, early-maturing variety of sweet corn is known to be wilt resistant. While the preliminary investigations here reported were conducted with dent corn, the results suggest the possibility of developing wilt-resistant sweet-corn varieties of high quality by means of the pure-line method of breeding.

<sup>1</sup> Received for publication Mar. 2, 1928; issued July, 1928. These investigations were conducted near Bloomington, Ill., in cooperation with Funk Bros. Seed Co.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 910.

## MATERIAL AND METHODS

Certain field experiments were undertaken in 1926 with a view to determining the relative resistance to bacterial wilt of a number of inbred lines and crosses of yellow dent corn, grown for another purpose. These experiments were conducted near Bloomington, Ill., on uniform, well-drained, productive brown silt loam soil. Two series of experimental plots, A and B, were located about 1 mile apart on adjoining farms where the soil conditions were similar.

In series A the corn was planted during the first week in May and in series B it was planted during the last week in May. The individual plots in series A included 45 plants, 15 of which were inoculated and 30 uninoculated. The individual plots in series B included 72 plants, 24 of which were inoculated and 48 uninoculated. No attempt was made to thin to a uniform stand either before or after inoculation.

The seed used included several inbred strains of yellow dent corn and their recombinations. The number of years of inbreeding ranged from five to nine. All strains matured in approximately the same number of days.

The organism used in the inoculations was isolated about four weeks preceding its use, from young Golden Bantam sweet-corn plants grown in Virginia in 1926.

The inoculations were made by means of a hypodermic syringe when the plants were from 24 to 30 inches high. Approximately 0.2 c. c. of a suspension of the bacteria in distilled water was injected into the central portion of each stalk about 2 inches above the crown of the plant. Previous experiments had shown that it was unnecessary to inject distilled water into the control plants (6). Yields of sound air-dried corn on the ear were used as the basis of judging the relative resistance of the different strains.

## RESULTS

Data on the resistance to bacterial wilt of 15 inbred strains of yellow dent corn and of 4 recombinations are given in Table 1.

Data in Table 1 show that reductions in yield of inbred strains of yellow dent corn, following inoculation with *Aplanobacter stewarti*, varied in series A from 5.8 per cent to 67.3 per cent. Unfortunately the strains that proved to be very high in resistance to bacterial wilt in series A, the earlier planting, had not been included in series B, but the strains that were intermediate in resistance in series A were also intermediate in series B. Strain 1 was very susceptible in both series. The greater reduction of strain 10 in series B was due largely to the fact that the three progenies used included only one of the more resistant ones. The appearance of this highly resistant progeny of strain 10, in contrast to one of the susceptible progenies of strain 1, is illustrated in Figure 1. It will be noted from Figure 1, A, that strains 10 and 1 were approximately equal in vigor. In most cases first-generation crosses were more resistant than their component inbred strains. It is interesting to note that the recombinations of strains 10 and 13 showed no reduction following inoculation, whereas the recombinations of strains 15 and 13 showed a reduction of 24 per cent following inoculation. These two crosses were comparable both in vegetative vigor and in date of maturity.





FIG. 1.—Inbred strains of yellow dent corn, uninoculated and inoculated with *Aplanobacter stewartii*: A.—a, strain 10, uninoculated; b, strain 1, uninoculated. B.—a, strain 10, inoculated; b, strain 1, inoculated

TABLE 1.—Differences in resistance to *Aplanobacter stewarti* of inbred strains in yellow dent corn, and crosses between them, as indicated by mean percentage reductions in yield of ear corn at Bloomington, Ill., in 1926, following artificial inoculation

Strain Nos. or F <sub>1</sub> from cross	Number of years of in-breeding	Number of progenies		Mean percentage reduction in yield		Rank in resistance
		Series A	Series B	Series A	Series B	
10×13			1		0	1
14×10			2		0.4 (±3.1)	2
2	9	6		5.8 (±2.0)		3
3	9	9		9.2 (±3.6)		4
5	9	9		9.2 (±3.6)		4
8×9			6		10.6 (±1.9)	5
13	9	39		12.9 (±2.4)		6
10	6	11	3	13.7 (±3.0)	28.2 (±16.2)	7
9	8		4		16.9 (±8.5)	8
4	9		5		20.7 (±2.0)	9
15×13			6		24.0 (±6.3)	10
6	9	7		24.0 (±5.7)		10
14	9	11		24.4 (±5.1)		11
12	5		22		26.0 (±1.6)	12
15	9		3		29.9 (±8.9)	13
11	9	7		38.8 (±4.4)		14
7	9		3		42.5 (±5.2)	15
8	9		5		51.6 (±8.9)	16
1	7	4	9	67.3 (±8.0)	68.4 (±3.3)	17

\* These progenies are included in those of the other series.

The data in Table 2 indicate that all the progenies of some inbred lines were very susceptible (strain 1), or resistant (strain 2), or uniformly intermediate (strain 4). Other strains, apparently homozygous for a number of visible plant characters, showed wide ranges in reaction to inoculation (strains 8, 13, and 14). However, in a strain as susceptible as strain 1 there was considerable variation in the extent of injury following inoculation. (Fig. 2.) The yield of progeny 1 of this strain was reduced 30 per cent by inoculation (fig. 2, B, a), but there was not a single sound ear in the inoculated portion of progeny 2 (fig. 2, B, b).

TABLE 2.—Differences in resistance to *Aplanobacter stewarti* in the various progenies in six inbred strains of yellow dent corn, as indicated by mean percentage reductions in yield of ear corn at Bloomington, Ill., in 1926, following artificial inoculation

Strain No.	Number of progenies	Mean percentage reduction in yield	Rank in resistance	Percentages of progenies with percentage reductions of—					
				1-5	6-10	11-25	26-50	51-75	76-100
2	6	5.8 (±2.0)	1	33	33	33	0	0	0
13	39	12.9 (±2.4)	2	41	8	20	28	3	0
4	5	20.7 (±2.0)	3	0	0	80	20	0	0
14	11	24.4 (±5.1)	4	18	10	18	36	18	0
8	5	51.6 (±8.9)	5	0	20	0	20	40	20
1	13	68.1 (±3.1)	6	0	0	0	15	62	23



FIG. 2.—Progeny rows of inbred strain 1 of yellow dent corn, uninoculated and inoculated with *Aplanobacter stewartii*: A.—a, progeny 1, uninoculated; b, progeny 2, uninoculated. B.—a, progeny 1, inoculated; b, progeny 2, inoculated

## SUMMARY

Bacterial wilt is economically important, especially on certain popular varieties of sweet corn.

The fact that the disease has been studied for 30 years without the losses being materially reduced indicates that no easy method of control is likely to be found.

Data are presented which show wide differences in reaction to inoculation with *Aplanobacter stewarti* in a number of inbred lines of yellow dent corn maturing in the same length of time. All the progenies of some inbred lines were uniformly high in resistance. These results suggest the possibility of developing resistance to bacterial wilt in some of the popular wilt-susceptible varieties of sweet corn. Other inbred lines, however, were less resistant and still others were uniformly susceptible.

There was no apparent correlation between resistance and vegetative vigor.

There was no apparent correlation between resistance to bacterial wilt and resistance to other important diseases, although strains with high wilt resistance usually were free from other diseases.

## LITERATURE CITED

- (1) McCULLOCH, L.  
1918. A MORPHOLOGICAL AND CULTURAL NOTE ON THE ORGANISM CAUSING STEWART'S DISEASE OF SWEET CORN. *Phytopathology* 8: 440-441, illus.
- (2) RAND, F. V.  
1923. BACTERIAL WILT OR STEWART'S DISEASE OF CORN. *Canner* 56 (10): 164-165.
- (3) ——— and CASH, L. C.  
1921. STEWART'S DISEASE OF CORN. *Jour. Agr. Research* 21: 263-264.
- (4) ——— and CASH, L. C.  
1924. FURTHER EVIDENCE OF INSECT DISSEMINATION OF BACTERIAL WILT OF CORN. *Science* (n. s.) 59: 67-69.
- (5) REDDY, C. S.  
1921. EXPERIMENTS WITH STEWART'S DISEASE ON DENT, FLINT AND SWEET CORN. (Abstract) *Phytopathology* 11: 31.
- (6) ——— and HOLBERT, J. R.  
1924. THE BLACK-BUNDLE DISEASE OF CORN. *Jour. Agr. Research* 27: 177-205, illus.
- (7) ——— HOLBERT, J. R., and ERWIN, A. T.  
1926. SEED TREATMENTS FOR SWEET CORN DISEASES. *Jour. Agr. Research* 33: 769-779, illus.
- (8) SMITH, E. F.  
1898. NOTES ON STEWART'S SWEET-CORN GERM PSEUDOMONAS STEWARTII, N. SP. *Amer. Assoc. Adv. Sci. Proc.* 47: 422-426.
- (9) ———  
1914. BACTERIA IN RELATION TO PLANT DISEASES. v. 3, illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 27).
- (10) ———  
1920. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. 688 p., illus. Philadelphia and London.
- (11) STEWART, F. C.  
1897. A BACTERIAL DISEASE OF SWEET CORN. *N. Y. State Agr. Expt. Sta. Bul.* 130, p. 423-439, illus.

# THE HOST RELATIONSHIP OF THE TREMATODE GENUS ZYGOCOTYLE<sup>1</sup>

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## HISTORICAL REVIEW

The occurrence of the same species of trematode in hosts as widely separate zoologically as birds and mammals is not common. A few such cases have been recorded for the Heterophyiidae, but so far as the writer is aware only one record exists in which an amphistome, normally a parasite of water birds, has been found in a mammal.

Diesing (2)<sup>2</sup> described an amphistome, *Amphistoma lunatum*, from the cecum of a ruminant, *Cervus dichotomus*, and also from the ceca of the following birds: *Anas melanotus*, *A. ipecutiri*, and *Himantopus wilsonii*. The specimens upon which his description was based had been collected several years previously by Natterer, in Brazil. Because of the wide difference in hosts, Dujardin (4) considered the record from *C. dichotomus* as probably a mistake, and later Diesing (3) apparently arrived at the same conclusion. Fischöder (5) re-described this species from some of the original material in the Vienna Museum, but was unable to find any essential differences between the mammalian and bird forms, and concluded that the citation of *C. dichotomus* as a host for this trematode was probably due to an error in labeling. Stunkard (7) transferred *A. lunatum* to the genus *Zygocotyle*, a genus proposed by him for a species of trematode, *Zygocotyle ceratosa*, found in the intestine of a duck, *Anas platyrhynchos*, from Nebraska.

Recently, through the courtesy of M. C. Hall, chief of the Zoological Division, the writer has been afforded an opportunity to study specimens of an amphistome reported by him (6) from the cecum of a cow, *Bos taurus*. These specimens were collected at Penonomé, Panama, June 10, 1926, and have been identified by the writer as *Zygocotyle lunata* (= *Amphistoma lunatum*). Aside from the fact that this constitutes a new host and geographical record for the species, it also apparently validates the previous doubtful record from *Cervus dichotomus*, and constitutes a second occurrence of this amphistome in a mammalian host.

The specimens, 30 in number, had been fixed in cold formaldehyde and many were much contracted. There is also considerable variation in size due to age, some being quite small and immature. The following description is based on 10 of the more mature specimens:

## DESCRIPTION OF ZYGOCOTYLE LUNATA FROM THE COW

Length, 4 to 6 mm.; width, 2 to 3 mm. The body form is oval and strongly curved ventrally (fig. 1), this curving being present in live specimens and perhaps exaggerated by preservation in a cold fixative. The color is yellowish white and

<sup>1</sup> Received for publication Mar. 19, 1928; issued July, 1928.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 914.

the cuticle is devoid of spines. The oral sucker is subterminal, strongly muscular, and is 390 to 650  $\mu$  in diameter. The oral evaginations or pharyngeal pouches are thick walled, 172 to 250  $\mu$  long and 117 to 130  $\mu$  wide, and open into the oral cavity near the origin of the esophagus. The acetabulum is large, strongly muscular, and opens ventrally. The opening is slightly wider than long, measuring 0.78 to 1.1 mm. by 0.91 to 1.2 mm. The posterior part of the acetabulum is cup shaped and the anterior part is conical, the apex being directed anteriorly and dorsally; the posterior edge is provided with a characteristic flap, which terminates on each side in a small conelike projection. The esophagus is 325 to 455  $\mu$  long, and is provided distally with a strong, muscular bulb 190 to 416  $\mu$  long by 130 to 260  $\mu$  wide. The intestinal ceca are thick walled and terminate at the level of the acetabular opening or a short distance cephalad of the opening. The testes are elongated transversely and lobulated or irregular in outline; in some of the smaller specimens the edges are almost smooth. The anterior testis measures 325 to 650  $\mu$  by 260 to 390  $\mu$ . The posterior testis lies immediately caudad of the anterior testis and measures 520 to 650  $\mu$  by 325 to 520  $\mu$ . The seminal vesicle is coiled and lies in front of the anterior testis. Cirrus sac absent. The ovary is oval or slightly irregular in outline, 156  $\mu$  by 260  $\mu$  in diameter, and lies about midway between the anterior part of the acetabulum and the posterior border of the posterior testis. In fully mature specimens, the uterus extends anteriorly in close coils to the genital opening, which is situated immediately caudad of the intestinal bifurcation. The vitellaria are composed of large

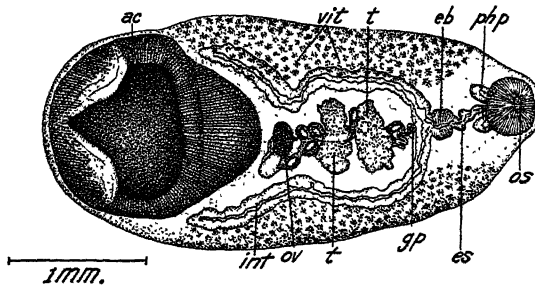


FIG. 1.—*Zygocotyle lunata*. Young specimen from cow; ventral view: ac, acetabulum; gp, genital pore; int, intestine; eb, esophageal bulb; es, esophagus; os, oral sucker; ov, ovary; php, pharyngeal pouch; t, testis; vit, vitellaria

follicles lying extracellally and extending from the level of the posterior end of the pharyngeal pouches to the level of the opening of the acetabulum. The eggs are oval, operculated, yellowish in color, and 130 to 143  $\mu$  long by 77 to 90  $\mu$  wide.

#### COMPARISON WITH SPECIMENS OF ZYGOCOTYLE FROM BIRD HOSTS

In view of the fact that Stunkard (7) considers the amphistome of ducks in North America as distinct from *Zygocotyle lunata*, the writer has compared the form described in this paper with amphistomes in the helminthological collection of the Bureau of Animal Industry, which had been collected from several bird hosts.

The material for this comparison consisted of: Six specimens collected by B. Schwartz from the domestic goose, *Anser anser domesticus*, at Washington, D. C., February 3, 1919; 1 specimen collected by E. A. Chapin from the redhead duck, *Marila americana*, at Bush River, Md., January 17, 1924; 6 specimens collected by the writer from the green-winged teal, *Nettion carolinensis*, at Wellborn, Tex., November 15, 1921; and 1 specimen from the jacksnipe, *Galinago delicata*, collected also by the writer at Wellborn, Tex., October 5, 1921.

TABLE 1.—Comparative measurements of specimens of *Zygocotyle* from the cow with those from various bird hosts

Name of host	Length of specimens	Width of specimens	Diameter of oral sucker	Pharyngeal pouch	Esophagus	Esophageal bulb	Anterior testis	Posterior testis	Ovary	Egg	Acetabulum
<i>Bos taurus</i> .....	<i>Mm.</i> 4-6	<i>Mm.</i> 2-3	$\mu$ 300-650	$\mu$ 172-250× 117-130	$\mu$ 325-455	$\mu$ 190-416× 130-280	$\mu$ 325-650× 200-390	$\mu$ 520-650× 325-520	$\mu$ 156-280	$\mu$ 130-143× 77-90	<i>Mm.</i> 0.78-1.1× .91-1.2
<i>Anser anser domesticus</i> .....	4-6	2-3	300-520	156-280× 130-195	455-650	195-156	680-900× 325-520	455-910× 286-520	175-325× 175-305	128-153× 170-96	.58-.66× .91-1.2
<i>Marila americana</i> .....	8	3	455	221×156	650	225×175	<sup>a</sup> 780	( <sup>a</sup> )	390×225	140×190	.78×.91
<i>Nettion carolinensis</i> .....	3-7	1.5-4	286-520	130-260× 65-208	260-520	167-260× 156-200	364-650× 221-520	390-585× 234-588	143-221× 143-195	125-160× 75-90	.76-.78× .6-.65
<i>Gallinago delicata</i> .....	3	1.5	286	130×65	280	169×136	364-221	390×234	143×91	125×75	.55×.62
<i>Zygocotyle ceratosa</i> (from Stunkard, 1917).....	3-6	1.45-2.4	370-530	160-220× 70-100	50-370	200-450× 180-230	200-300	550-780	200-300	140×83	1.1×.74
<i>Zygocotyle lunata</i> from (Fischneider, 1903).....	5-9	1.6-3	---	300-350× 100-120	1,000-1,300	300×300	500	---	300	145-150× 72-75	.8-1×1-1.5

<sup>a</sup> Obscured by the greatly distended uterus.

Observations on these specimens showed them to agree in all essential characters, and they are therefore considered to be of the same species. Considerable variation was found in the measurements, but as there was no great disparity in the shape and relative position of the different organs, these differences are not regarded as significant, especially since as great variation was found in specimens from the same host as in those from the different hosts. A rather remarkable difference was observed in the egg sizes of the different specimens, the variation being in some instances as much as  $25\ \mu$  in the length and  $21\ \mu$  in the width. These differences are similar to those reported by Cort (1) for other trematodes.

Table 1 gives a comparison of the measurements obtained, and for further comparison the measurements given by Stunkard (7) for *Zygocotyle ceratosa* and by Fischoeider (5) for *Z. lunata* are included.

It will be noted in this table that for the most part the measurements of the specimens studied by the writer intergrade with those given for *Zygocotyle ceratosa* and *Z. lunata*. In view of this fact, and in the absence of consistent morphological differences, it is the opinion of the writer that the form occurring in ruminants is the same as that occurring in birds, and that the recognition of *Z. ceratosa* as a distinct species is not justified. For the time being *Z. ceratosa* is regarded as a synonym of *Z. lunata*.

#### SUMMARY

A species of amphistome, *Zygocotyle lunata*, apparently normally parasitic in water birds, is reported for the first time in a domestic ruminant, *Bos taurus*. This record is the second report of this species from ruminants, the first being from a deer, *Cervus dichotomus*.

The following new bird hosts are reported for this trematode: *Anser anser domesticus*, *Gallinago delicata*, *Marila americana*, and *Nettion carolinensis*.

A comparison of specimens from the cow with specimens from water birds indicates that they are specifically identical, and that the recognition of *Zygocotyle ceratosa* as a distinct species is not justified. *Z. ceratosa* is, therefore, regarded as a synonym of *Z. lunata*.

#### LITERATURE CITED

- (1) CORT, W. W.  
1915. EGG VARIATION IN A TREMATODE SPECIES. Jour. Parasitol. 2: [25]-26.
- (2) DIESING, C. M.  
1836. MONOGRAPHIE DER GATTUNGEN AMPHISTOMA UND DIPLODISCUS. Ann. Wiener Mus. Naturgesch. 1: [235]-260, illus.
- (3) ———  
1850. SYSTEMA HELMINTHUM. v. 1. Vindobonae.
- (4) DUJARDIN, F.  
1845. HISTOIRE NATURELLE DES HELMINTHES OU VERS INTESTINAUX. 654 p., illus. Paris.
- (5) FISCHOEIDER, F.  
1903. DIE PARAMPHISTOMIDEN DER SÄUGETHIERE. Zool. Jahrb., Abt. System., Geogr. u. Biol. Thiere 17: [485]-660, illus.
- (6) HALL, M. C.  
1927. THE PARASITE PROBLEMS OF THE LIVE STOCK INDUSTRY IN THE UNITED STATES AND IN CENTRAL AMERICA. Jour. Amer. Vet. Med. Assoc. (n. s. 23) 70: 935-946.
- (7) STUNKARD, H. W.  
1917. STUDIES ON NORTH AMERICAN POLYSTOMIDAE, ASPIDOGASTRIDAE, AND PARAMPHISTOMIDAE. Ill. Biol. Monog., v. 3, no. 3, 114 p., illus.



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## EXPERIMENTS ON THE ERADICATION OF CANADA THISTLE, *Cirsium arvense*, WITH CHLORATES AND OTHER HERBICIDES<sup>1</sup>

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### INTRODUCTION

Canada thistle, *Cirsium arvense* (L.) Scop., is a perennial weed. Its root system penetrates deep into the ground, survives the winter, and forms shoots the following spring. Canada thistle propagates very rapidly and is difficult to eradicate. The experiments herein reported were undertaken in order to develop methods for its eradication with herbicides on arable land.

### HISTORICAL REVIEW

The use of herbicides on arable land is a relatively new achievement. The destruction of annual weeds in grain fields was first attempted about 1895, as has been stated elsewhere (6).<sup>3</sup> This method is now rather extensively used in some countries. When it was desired to destroy perennial weeds on arable land, the herbicides were either applied to single plants or repeated sprayings were necessary. Thompson and Robbins (31) reported good results from the application of several herbicides to barberry bushes, *Berberis vulgaris* L. Gray (16), Stewart and Pittman (30), and other workers have reported experiments in which herbicides, generally sodium arsenite, was sprayed on perennial weeds. Two or more applications were found necessary for eradication. A practical method for the destruction of perennial weeds on arable land by a single application of a herbicide has not yet been described.

In the following experiments the use of chlorates gave the most satisfactory results. The earliest record of the use of chlorates for the eradication of weeds that has come to the writer's attention dates back to 1901. Potassium chlorate was applied to prickly pear in Australia (1). The results seem to have been unsatisfactory. A considerable time elapsed before they were mentioned again, but during the last five or six years they have been tested rather extensively. The new experiments seem to have begun in France. Loyer (21) reports some experiments in 1923. However, he mentions the fact that Rabaté recommends the application of 250 kgm. of sodium chlorate per hectare in a 2 per cent solution for the destruction of all vegetation on garden paths, etc.; so that some experiments must have been performed earlier. Loyer (21) recommends an application of

<sup>1</sup> Received for publication Apr. 6, 1928; issued August, 1928.

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 332.

800 liters of 1 per cent solution of ammonium chlorate ( $\text{NH}_4\text{ClO}_3$ ) in grain crops for the destruction of annual weeds. He adds that an application of 25 kgm. per hectare will not injure the grain. Early in 1925 recommendations based on experiments "during the last three years" were published in Denmark (29). A 5 per cent solution of sodium chlorate applied at the rate of 6,000 to 8,000 liters per hectare is recommended for the eradication of weeds on garden paths, etc. A 1.5 to 2 per cent solution, at the rate of 1,000 liters per hectare, was recommended for the destruction of annual weeds in grain crops. Korsmo (18, 19) reports experiments during 1923-1926. In his latest report (19) he states that an application of a 5 per cent solution of sodium chlorate at the rate of 1 liter per square meter killed 11 species of perennial weeds, including Canada thistle. Eight perennial weeds, including quack grass, *Agropyron repens* (L.) Beauv., were not killed by a single application. The experiments were performed on weeds growing along ditches, roadsides, etc. Feilitzen (11) recommends a 5 per cent solution of sodium chlorate applied at the rate of 1 liter per square meter for the eradication of all vegetation on tennis courts, etc. In 1924 and 1925 the writer (3, 4) used dilute solutions of sodium and potassium chlorate for the eradication of annual weeds in grain crops. The sprays were found to injure the grain almost as much as the weeds. Latshaw and Zahnley (20) obtained promising results by using a 12.5 per cent solution of sodium chlorate spray on field bindweed, *Convolvulus arvensis* L. The optimum time for the first application of spray seemed to be about the time the flowers were in full bloom.

#### THE WORKING HYPOTHESIS

Eradication of perennial weeds must aim at the destruction of the perennial structures. The destruction of the annual growth weakens the plants. Repeated destruction may lead to eradication, as the perennial part becomes exhausted by the forced shoot production. The poisoning of the perennial parts with a herbicide will kill the whole plant.

The perennial part of Canada thistle is its root or root system, the principal part of which consists of the propagation roots which grow more or less horizontally 15 to 30 cm. below the surface of the ground. These roots form numerous shoots.

The poisoning of the root system of Canada thistle may be assumed to be possible in two ways. Herbicides may be applied to the tops or to the roots. It has been found, however, that herbicidal sprays applied to the tops of plants do not easily penetrate into the roots. Schulz and Thompson (28) dipped tops or leaves of tomato plants in an arsenical spray solution. It was found that the treated parts died, but the arsenic moved only slightly or not at all to adjacent tissues of the plants. Barberry bushes were not killed when the spray was applied to the foliage. This fact indicates that the poison was not transported to the roots. Gray (15) found that spraying mature vines of morning glory within the fog belt with an arsenical spray killed the roots to a depth of 3 to 4 feet below the surface of the ground. Gray (16) as well as other workers have failed to obtain such good results under less humid conditions. Some work by Crofts<sup>4</sup> indicates that the reaction of the spray may influence the extent

<sup>4</sup> Oral communication of unpublished data by A. S. Crofts, University of California. 1927.

which it will penetrate the plants. He has found arsenic to a maximum depth of 60 cm. in roots of morning glory when the vines were sprayed with an acid arsenical spray. A considerable number of references dealing with the application of herbicidal sprays on perennial weeds show that a single spraying of the top growth generally causes but little damage. Repeated sprayings have been found necessary. For instance, Munn (24) found it necessary to spray dandelions, *Taraxacum officinale* Weber, three to five times during the summer with an 18 to 25 per cent iron sulphate solution in order to obtain eradication or even control. Detmers (10) found that single applications of either calcium arsenite or sodium arsenite sprays were insufficient to exterminate Canada thistles from a pasture. These chemicals did not seem to penetrate to the underground parts of the weeds.

Schulz and Thompson (28) found that several plants were killed rapidly by the application of sodium arsenite solutions to their roots. Arsenic was found throughout the plant tissues. Also other experiments show that solutions are more easily carried from the roots toward the top than in the reverse direction. Thus, the application of a poison to the roots seems to be a more reliable method for the eradication of perennial weeds than spraying the tops.

The application of a herbicide to the root system of Canada thistle on arable land involves a consideration of several factors.

1. The herbicide must penetrate the soil freely, for the propagation roots of Canada thistle grow at a depth of 15 to 30 cm.

2. The herbicide must decompose or leach out of the soil in order not to interfere with the normal crop production.

3. The herbicide must be effective in relatively small quantities.

The penetration of chlorates, the herbicides to be tested, seemed to be rather good. Feilitzen (12) found that coltsfoot, *Tussilago farfara* L., was killed by an application of a 5 per cent solution of sodium chlorate at the rate of 1.25 liters per square meter. This weed has rootstocks which penetrate the ground to a great depth. Since chlorates are rather unstable compounds, it can be assumed that they disappear rapidly in the soil. Loyer (21) found that an application of 45 kgm. of ammonium chlorate per hectare did not harm the succeeding crop. Several workers have reported that chlorates are very effective for the destruction of vegetation on tennis courts, etc. In view of these findings, chlorates seemed to offer promising results as herbicides. However, there appears to be a great discrepancy in the reports of the sensitiveness of the plants to chlorates. In France (21) an application of a 2.5 per cent solution of ammonium chlorate for the eradication of annual weeds in grain crops was claimed to be noninjurious to the grain plants. In Denmark (29) a 2 per cent solution was recommended for that purpose. The writer (3, 4) found a 1 per cent solution to be very injurious to barley in northern Sweden. In all the above trials the amount of the spray was 1,000 liters per hectare and the application was made to spring-sown grain crops. This seemed to indicate that there was a gradient in the action of the chlorates. It appeared that temperature greatly influenced their action, as they seemed to be more active the farther north they were tried.

The amount of chlorates used for the destruction of all vegetation on tennis courts, etc., is generally large. Korsmo (19) used 300 to

500 kgm. of sodium chlorate per hectare. Feilitzen (12) applied 500 to 1,250 kgm. These applications are heavy enough to prevent the use of chlorates on arable land. The cost would be prohibitive. However, if the effect of the chlorates is greater with low temperature, smaller applications late in the autumn or early in the spring might be found sufficient to eradicate Canada thistle.

There were still other indications of the possibility that small quantities of chlorates would have the desired result, if the application were made at the right time: (1) The penetration through the soil might be facilitated; (2) the root system of Canada thistle might be less resistant during certain seasons than others.

The penetration of the herbicide should take place more rapidly when more water is sinking into the soil. The application of the herbicide on wet soil receiving additional water in the form of precipitation would promote the penetration of the herbicide to the roots of Canada thistle. During late autumn and early spring such climatic conditions may prevail within humid regions.

The work of Lund and Rostrup (22) and Paczosky (25) shows that a part of the root system of Canada thistle dies during the winter. Thus the application of a poison at that time might be more effective than when plants are in full vigor during the vegetative period. The repeated freezing and thawing of the soil during the winter is another strain on the roots, and a small quantity of a poison might prove fatal to the roots during the prevalence of conditions so unfavorable for the plant.

It is obvious that a herbicide, to be used on arable land, must harm the crop plants to the least possible degree, if it shall be of any practical value. All fields to be spring sown are bare in autumn. An application of herbicides at that time will give the poison a long time to act before any crop is sown. During the winter the herbicide might leach out of the soil or decompose, so that no injury would be caused to the spring-sown crop.

These considerations prompted the writer to undertake the experiments herein reported. Chlorates (and other herbicides for comparison) were applied in the autumn and at various seasons during the year for the eradication of Canada thistle.

## EXPERIMENTAL DATA

### PRELIMINARY EXPERIMENTS

A preliminary field experiment started in the autumn of 1925 has been reported previously (5). Solutions of sodium chlorate,  $\text{NaClO}_3$ , potassium chlorate,  $\text{KClO}_3$ , and sodium arsenite,  $\text{NaHAsO}_3$ , were sprayed on the ground after frost had killed the tops of the vegetation. The applications were made on permanent grasslands infested with Canada thistle. By the following spring the chlorates had severely injured or eradicated the thistles while the arsenite had been without effect. Figure 1 illustrates the effect of sodium chlorate on Canada thistle.

In order to select additional herbicides to be tested with the chlorates some experiments were conducted during the winter of 1925-26. Cuttings of roots of Canada thistle were planted in pots. When shoots sprouted oats were sown in the pots and when the oat seedlings were about 10 cm. high solutions of herbicides were applied

at the rate of 100 to 500 p. p. m. (parts per million) of dry soil. The following chemicals were used: Sodium chlorate,  $\text{NaClO}_3$ ; potassium chlorate,  $\text{KClO}_3$ ; sodium perchlorate,  $\text{NaClO}_4$ ; potassium sulphide,  $\text{K}_2\text{S}$ ; calcium sulphide,  $\text{CaS}_2$ ; sodium cyanide,  $\text{NaCN}$ ; and sodium thiocyanate,  $\text{NaCNS}$ . It was found that cyanide killed the thistles and oats in 3 days, the thiocyanate killed the plants in 12 to 18 days, depending on the amount applied, while the chlorates killed the thistles in 14 to 20 days. Amounts of 300 p. p. m. or more killed the



FIG. 1.—Photographs showing the effect of sodium chlorate on Canada thistle growing in grassland. A, thistle from control plot; B, thistle from plot treated with 150 kgm.; and, C, thistle from plot treated with 200 kgm. per hectare, on November 11, 1925. On plots treated with larger quantities no thistles appeared the following year. Thistles harvested and photographed on June 30, 1926

oats as well as the thistles. The perchlorate, in amounts of 400 p. p. m. or more, killed the thistles and oats in 40 days. Other herbicides applied had no appreciable effect on the plants.

#### SECOND FIELD EXPERIMENT

##### THE APPLICATION OF HERBICIDES AT VARIOUS SEASONS

In the first field experiment it was found that chlorates applied on the ground late in autumn eradicated Canada thistle before the following spring, and in pot experiments sodium cyanide and thiocyanate were found more poisonous to the thistle than the chlorates. In order to compare these herbicides under field conditions, the following experiment was conducted.

In June, 1926, a field on the farm of the New York State College of Agriculture, Ithaca, N. Y., was secured for the experiment. This

field had been sown to oats and was heavily infested with Canada thistles. The soil was of the Dunkirk silty clay-loam type, rather poor in organic matter and with a reaction of  $P_H$  5.3. The subsoil was very compact and had poor drainage. Plots 10 square meters in area were laid out. A diagram of these plots is shown in Figure 2. On June 22 the number of thistles was determined on each plot. The results are recorded in Tables 1 to 4. It was decided to apply the herbicides not only in different amounts but also at different seasons. The first application was made on June 28; the second on September

1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81	82	83	84
85	86	87	88	89	90	91	92	93	94	95	96

FIG. 2.—Diagram of plots in second field experiment. The plots were nearly square and were separated by paths 1 meter wide.

1, just after the oats were harvested; the third on November 1; and the last on March 30 the following spring. Only one plot for each treatment was possible, as five different amounts of each herbicide were used at each time and the field was of limited area. The herbicides were applied dry and broadcast as evenly as possible. The following commercial products were used: Sodium chlorate,  $NaClO_3$ ; potassium chlorate,  $KClO_3$ ; sodium cyanide,  $NaCN$ ; and sodium thiocyanate,  $NaCNS$ . The quantities employed were from 100 to 300 kgm. per hectare. The applications were made in accordance with a certain scheme which distributed the treatments evenly over the experimental field. One-sixth of the plots were left untreated. The amounts applied to each plot are recorded in Tables 1 to 4. At intervals the effect of the herbicides on the vegetation was noted.

TABLE 1.—Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied June 28, 1926

Plot No.	Treat- ment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
<i>Na CN</i>					
24.....	100	112	56	1, 250	1, 320
14.....	150	84	77	1, 520	1, 600
9.....	200	198	162	1, 320	1, 580
4.....	250	101	15	1, 380	1, 380
19.....	300	82	92	1, 290	1, 330
<i>KClO<sub>3</sub></i>					
2.....	100	7	15	1, 940	2, 200
22.....	150	159	5	1, 240	1, 220
17.....	200	128	7	1, 270	1, 260
12.....	250	120	0	1, 360	1, 210
7.....	300	164	2	1, 200	1, 100
<i>Na ClO<sub>3</sub></i>					
8.....	100	140	19	1, 640	1, 930
3.....	150	43	1	1, 240	1, 250
23.....	200	157	0	1, 310	1, 230
18.....	250	82	1	1, 040	900
13.....	300	49	0	1, 270	1, 420
Controls untreated (average).....		124	108	1450±126	1670±142

TABLE 2.—Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied September 1, 1926

Plot No.	Treat- ment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
<i>Na CN</i>					
27.....	100	92	191	1,910	2,830
31.....	150	87	141	1,600	1,920
20.....	200	131	160	1,220	1,720
11.....	250	132	201	1,000	1,520
34.....	300	94	162	1,460	1,830
<i>Na CNS</i>					
35.....	a 100	101	96	1,200	1,600
36.....	a 150	44	43	1,390	1,710
37.....	a 200	172	98	1,420	1,930
39.....	a 250	158	108	2,230	3,160
42.....	a 300	160	84	1,640	3,210
<i>KClO<sub>3</sub></i>					
5.....	100	178	32	1,360	1,480
28.....	150	15	12	1,660	2,170
25.....	200	120	2	1,310	1,460
15.....	250	48	18	1,240	1,200
32.....	300	172	0	850	760
<i>Na ClO<sub>3</sub></i>					
16.....	100	59	38	1,080	1,040
10.....	150	162	39	1,020	1,000
30.....	200	141	0	1,240	1,320
26.....	250	162	1	1,520	1,900
33.....	300	168	0	560	630
Controls untreated (average).....		105	83	1,510±112	1,730±126

a Applied September 25.

TABLE 3.—Number of shoots of Canada thistle on plots 10 square meters before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied November 1, 1926

Plot No.	Treat- ment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
<i>Na CN</i>					
43.....	100	121	111	1,740	2,100
62.....	150	162	140	1,500	a 2,800
58.....	200	106	118	1,570	2,230
51.....	250	132	201	1,700	2,480
47.....	300	48	96	1,490	1,920
<i>Na CNS</i>					
56.....	100	111	152	2,080	a 3,500
50.....	150	140	182	1,780	a 3,660
46.....	200	102	71	1,440	2,180
67.....	250	104	86	1,460	2,000
61.....	300	112	121	1,480	2,600
<i>KClO<sub>3</sub></i>					
44.....	100	113	56	1,700	1,940
63.....	150	90	92	1,540	1,770
59.....	200	43	6	1,400	1,760
54.....	250	148	1	1,360	1,520
48.....	300	28	4	1,350	1,520
<i>Na ClO<sub>3</sub></i>					
49.....	100	151	68	1,400	a 2,400
45.....	150	190	6	1,160	1,160
66.....	200	64	0	1,260	1,040
60.....	250	28	1	1,200	1,160
55.....	300	86	0	1,300	1,040
Controls untreated (average).....		71	142	1,360±86	a 2,650±83

a Straw yields include thistles.

TABLE 4.—Number of shoots of Canada thistle on plots 10 square meters, before and after treatment with herbicides, and yield of oats in kilograms per hectare; herbicides applied March 30, 1927

Plot No.	Treat- ment (kgm. per hectare)	Number of thistle shoots		Yield of oats, Aug. 13, 1927 (kgm. per hectare)	
		June 22, 1926	Aug. 13, 1927	Grain	Straw
<i>Na CN</i>					
73.....	100	33	61	1,320	2,000
93.....	150	73	42	890	700
87.....	200	5	43	1,520	<sup>a</sup> 2,370
83.....	250	39	26	1,080	1,320
79.....	300	99	87	1,940	2,380
<i>Na CNS</i>					
91.....	100	64	64	1,370	1,500
86.....	150	6	72	1,750	<sup>a</sup> 2,700
82.....	200	81	48	460	520
77.....	250	68	78	200	340
96.....	300	5	20	0	0
<i>KClO<sub>3</sub></i>					
80.....	100	61	78	0	0
75.....	150	0	17	0	0
94.....	200	100	12	0	0
89.....	250	62	52	0	0
84.....	300	13	0	0	0
<i>Na ClO<sub>3</sub></i>					
85.....	100	60	65	410	380
81.....	150	76	6	0	0
76.....	200	13	4	0	0
95.....	250	64	7	0	0
90.....	300	39	15	0	0
Controls untreated (average).....		29	62	1,310±57	1,830±73

<sup>a</sup> Straw yields include thistles.

Cyanide applied on June 28 rapidly killed the surface vegetation of the plots. New thistle shoots appeared soon afterwards, indicating that the roots were unharmed. The application made on September 1, or just after the oats were harvested, did not injure the vegetation. Seedlings of oats, ragweed, *Ambrosia artemisiifolia* L., yellow foxtail, *Setaria glauca* (L.) Beauv., and shoots of Canada thistle grew vigorously during the autumn, indicating that the nitrogen of the cyanide had been available as plot nutrients. The application on November 1 killed the vegetation within a fortnight.

The thiocyanate was not applied before September 25. Its application at that date had a marked influence on the vegetation. The larger amounts killed the annual vegetation and harmed the thistles considerably. Similar effects were noted after the application on November 1.

The effect of the chlorates applied in June was not very noticeable. The oats continued to grow without injury and the thistles became only a little scorched. The effect of the potassium chlorate was less apparent than that of the sodium chlorate, probably owing to lower solubility. This inhibition of the effect of the chlorates may be explained as due to lack of rain. The salts did not dissolve, or at least did not penetrate to the absorbing parts of the roots of the plants. However, when the oats were harvested, and the new vegetation appeared on the untreated plots, the chlorate plots remained bare of annual plants. The poisons prevented seeds from germinating or



killed the seedlings. The thistles were less injured. Some leaves were scorched, but in other ways they were growing almost as well as on the untreated plots. The application of chlorates on September 1 had a similar effect on the vegetation, for rain dissolved the salts rather soon. The chlorates applied on November 1 killed the annual plants in about three weeks, but the thistles were not seriously harmed. There was no difference in the action of potassium and sodium chlorate.

The field was not plowed in the autumn. On May 7, the plots were disked three times, fertilizers broadcast over the field, and Early Dakota oats sown. The cold weather during May checked the growth, so that the oats were rather weak when the drought in June set in. As a result the crop yields were rather low. The oats were harvested on August 13 and threshed. The results, which are recorded in Tables 1 to 4, show that the yields vary considerably. The probable error of untreated plots is large. There is a clear detrimental effect of chlorates and thiocyanate applications that were made in the spring, five weeks before the oats were sown. Thiocyanate applied the preceding year had no effect on the yield of oats, while the highest applications of chlorates in some instances decreased the yields. The decrease in yields of straw is, however, in most instances due to decrease in number of thistles. The sodium cyanide in several cases shows a distinct fertilizing effect.

The number of thistles on the plots were counted several times during the summer of 1927; the numbers found on August 13, when the plots were harvested, are recorded in Tables 1 to 4. On the cyanide and thiocyanate plots there was no influence of the herbicides on the thistles. On the chlorate plots, on the contrary, the thistle shoots failed to appear or were found late in the season.

#### EXPERIMENT TO DETERMINE PENETRATION OF HERBICIDES THROUGH SOIL

Of the various herbicides used in the first and second field experiments only the chlorates had eradicated the thistles. The preliminary pot experiments had shown sodium cyanide and thiocyanate to be more toxic than chlorates to the Canada thistle. Work reported in literature shows that soluble arsenite had no effect on the Canada thistle. As the herbicides were applied on the surface of the ground, while the horizontal "propagation roots" of the thistle were found at a depth of 15 to 20 cm., it is clear that the herbicides must penetrate the soil to that depth in order to affect the plants. The difference in action between the herbicides tested may for that reason be due partly or wholly to different rates of penetration through the soil in the field as compared with soil in pots. Some experiments were performed in order to determine if the herbicides penetrate a soil at different rates and if penetration is influenced by soil type. The penetration of solutions of herbicides through columns of soil was determined. However, in order to be sure that the values obtained represented the penetration of the herbicides through soil special cylinders were made for the soil columns.

#### APPARATUS USED

If the penetration of a solution through soil is measured when the soil is kept in containers such as cylinders of glass, metal, etc., the values obtained may be influenced by the nature of the cylinder. A

solution may flow more easily along the wall of the cylinder than through the soil. Absorption or adsorption of solution by the wall of the cylinder may influence the results. In order to avoid these sources of error cylinders were made of fine mesh wire netting and coated with paraffin as follows: Cylinders were made of wire netting with meshes about 3 mm. 35 cm. in height, and with a cross section area of 1 sq. decim. A bottom of the same netting was inserted 5 cm. from the lower end and the edges of the cylinder soldered together. Thereafter the cylinder was dipped in melted paraffin until a thin coating was formed. A filter paper was placed in the bottom of the cylinder and dry soil, passed through a sieve (20 meshes per inch), was added with uniform packing to a height of 15 cm. The paraffin coating the part of the cylinder that was filled with soil was then melted. The melting paraffin was absorbed by the soil. The cylinder, while still hot, was dipped several times in melted paraffin until a rather thick coating was formed. Enough water was then added to saturate the soil, and the cylinder was left standing until the next morning, when the water was found to have reached the bottom of the soil column. The paraffin on the bottom of the cylinder as well as the filter paper was now perforated at every mesh of the netting, so that the water could flow through. The wet soil did not pass through these holes.

Two sets of cylinders were made. The first set was filled with Dunkirk silty clay loam collected from the field on which the second field experiment was located. The reaction of the soil was  $P_H$  5.77 as measured by the quinhydrone electrode. As the reaction of the soil might have some influence on the penetration of the herbicides, a second set of cylinders was made and filled with Lansing silt loam which had a reaction of  $P_H$  7.87. Each set was made up of four cylinders as the penetration of four herbicides—namely, sodium chlorate,  $NaClO_3$ ; sodium thiocyanate,  $NaCNS$ ; sodium cyanide,  $NaCN$ ; and sodium arsenite,  $NaHAsO_3$ —was to be determined. The cylinders were mounted on Büchner funnels, filled with water, and left for three days in order that the soil might become saturated. The penetration of water was measured and compared with the penetration of the herbicidal solutions later on.

At the outset of the experiment, the cylinders were emptied of water and 1 liter of N/10 solutions of the herbicides was added. The amount of solution in the cylinders was kept constant by daily addition of amounts equal to those leaching through the soil. The amount of solution leaching through the soil was recorded daily and analyzed for the herbicides.

#### ANALYTICAL METHODS

The analysis for chlorates was performed according to the method of Treadwell and Hall (32). A sample of the solution to be analyzed was transferred to an Erlenmeyer flask fitted with a Bunsen valve. The air was expelled with a stream of carbon dioxide. A definite amount of an acid iron sulphate ( $FeSO_4$ ) solution was added, and the mixture boiled. Chlorates oxidize the iron sulphate to  $Fe_2(SO_4)_3$  and the excess of  $FeSO_4$  was determined by titration with a potassium permanganate solution.

Analyses for thiocyanate were made colorimetrically. To a given amount of the solution (leaching), 24 c. c. in the first tests and 1 c. c. in the last, 1 c. c. of a normal  $Fe_2(SO_4)_3$  solution was added. The

amount was made up to 25 c. c., filtered, and the color compared with a standard made up of 24 c. c. N/500 NaCNS plus 1 c. c.  $\text{NFe}_2(\text{SO}_4)_3$  solution. A Duboscq colorimeter was used.

The analyses for cyanide were made according to the Official Methods (7). Ten cubic centimeter portions of the solution were titrated with a N/50  $\text{AgNO}_3$  solution until a faint turbidity was noticed.

The analyses for arsenic were made according to the method used by Schulz and Thompson (28). The essential feature of the analysis is that the evolving arsine is caught in a 5 per cent solution of mercuric chloride ( $\text{HgCl}_2$ ) and determined by titration with sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ).

#### RESULTS

Chlorates appeared in the solution leaching through the Dunkirk silty clay loam after five days, and in the leachings from the Lansing silt loam after three days. The thiocyanate was found in leachings from the former soil after four days and in leachings from the latter soil after three days. Cyanides were not found to penetrate either soil. Analyses were made of leachings from Dunkirk soil for 40 days and for the Lansing soil for 30 days. Arsenic was found in a concentration of N/1000 in leachings from the Dunkirk soil after 34 days, increasing to about N/500 10 days later. The solution from the Lansing soil gave tests for arsenic after 18 days; on the thirtieth day the concentration in the leachings was N/400.

The chlorate and thiocyanate had no noticeable influence on the soil in the cylinders. The amount of solution leaching through remained constant and was uncolored throughout the experiment. The cyanide and arsenite solutions had a dissolving action on the organic matter of the soil. The supernatant liquid in the cylinders became brown and even the solutions leaching through soon were of a brownish color. These solutions also made the soils less permeable. The amount of solution leaching through the soil columns decreased to about one-third in 21 days, the period during which the leachings were measured daily.

The curves in Figure 3 illustrate the leaching of sodium chlorate and sodium thiocyanate through Dunkirk silty clay loam. It is seen that they penetrate freely. After 18 days the concentration of the solution leaching through a soil column of 15 cm. was found to be equal to the concentration of the solution added. The similarity of the curves indicates that the salts probably do not react with any substances in the soil.

A solution of sodium cyanide is very alkaline. Its slow penetration is probably, in part at least, due to this fact. When the above experiments were concluded, the penetration of a solution of sodium cyanide which was titrated to neutrality was determined. It was found to leach through the Dunkirk soil column in 22 days. The amounts were very small.

#### EXPERIMENT TO DETERMINE DECOMPOSITION OF HERBICIDES IN THE SOIL

A preliminary experiment showed that sodium cyanide and thiocyanate disappear rather soon from soil kept in a greenhouse. If there is a rapid disappearance it may prevent their action in field experiments. However, the decomposition may be assumed to proceed more rapidly in soil kept in a greenhouse of high temperature and at optimum moisture content than in a field. If the temperature

is found to play an important part in the decomposition of the herbicides their application late in autumn or early in spring on ground of low temperature may give the desired results.

The following experiment was conducted in order to study the influence of temperature on the decomposition of the herbicides. Ordinary glass tumblers were filled with 200 gm. of moist soil collected in a field in the beginning of April. This moist field soil was preferred to the dry greenhouse soil as the catalytic power of the soil in some way might influence the decomposition of the herbicides. The catalytic power is known to diminish very markedly when the soil is dried. The moisture content of the soil was 24.9 per cent; so that each tumbler contained 160.2 gm. of dry soil. The tumblers were covered with Petri dishes and placed in the dark in incubators at 10°, 20°, and 30° C. A week later 10 c. c. of a solution containing 16 mgm. of herbicide was added to each tumbler, thus making the concentration in the tumbler 100 p.p.m. of dry soil. The solutions

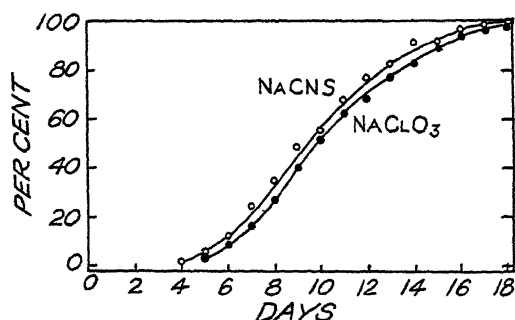


FIG. 3.—Penetration of sodium thiocyanate and sodium chlorate through Dunkirk silty clay loam. After 18 days the concentration of the solution leaching through a 15 cm. soil column was equal to the concentration of the solution added

were well mixed with the soil. Sodium chlorate, sodium thiocyanate, and sodium cyanide were the herbicides applied. Fifteen tumblers were left untreated for controls.

One tumbler of each series was planted each week for 10 weeks. As the chlorates were expected to decompose more slowly than the other herbicides, especially at lower temper-

ature, 15 tumblers containing that herbicide were kept at 10° C. Controls from the three temperatures used were planted every other week. As the experimental plant Alpha barley was used. Ten seeds were planted and five seedlings allowed to grow in each tumbler. The seeds were planted in the first series of tumblers on April 9, 1927, one week after the herbicides were applied. After the seeds were planted, the tumblers were kept in the incubators until the seedlings were 3 to 4 cm. high. The tumblers were then moved to a greenhouse. Notes on the height and appearance of the plants were taken on removal to the greenhouse and thereafter at weekly intervals.

It was found that sodium cyanide decomposed so rapidly even at low temperatures, that the plants grew better in tumblers treated with that herbicide than in the controls. The thiocyanate decomposed so rapidly in tumblers kept at 20° and 30° C. that it had no poisonous effect on the plants. At 10° this herbicide did not decompose rapidly enough to leave unaffected the first barley planted. The plants sown one week after the poison was applied died at an average height of 4.5 cm. Plants sown one week later attained an average height of 12 cm. Plants from seeds sown later were somewhat stunted in the early stages of growth but recovered and grew to full size. The sodium chlorate had a much more lasting effect. In tumblers kept at 10° the barley seedlings died rapidly. The plants

from the first sown barley reached an average height of 2 to 3 cm.; barley sown up to 15 weeks after the herbicide was applied reached a height of 8 to 10 cm. Plants in the tumblers kept at 20° attained a larger size before dying, especially in those sown later. The difference was still more marked in tumblers kept at 30°. In these the seedlings grew to about 10 cm. in the first sown tumblers, and in the one sown 10 weeks after the herbicide was applied the plants seemed to grow to full size. When the uninjured plants had reached a height of about 30 cm. they were discarded. The difference in the size of the plants reached before death probably did not give a true indication of the decomposition of the chlorate. In the tumblers kept at the higher temperatures the seedlings grew very fast, so that they had reached a larger size before the herbicide had had time to act. The time necessary for the chlorate to kill the seedlings was about equal for all temperatures in the beginning of the experiment. The seedlings died after growing for three weeks in the greenhouse. In the latter part of the experiment, however, the difference in time was striking. In tumblers kept at 10° the plants died almost as soon as in the beginning of the experiment, or in about three weeks in the greenhouse, while in the 20° tumblers they were growing for six weeks in the last-planted tumbler, and in the tumbler kept at 30° for ten weeks they seemed to grow indefinitely. Thus the action of the chlorate diminished with time, especially at higher temperatures. This seems to have been due to the decomposition of the chlorate as no leakage was possible.

In Table 5 and in Figure 4 the main effects of sodium chlorate upon barley seedlings are shown. The effect of sodium cyanide and thiocyanate are not given as these herbicides disappeared so rapidly.

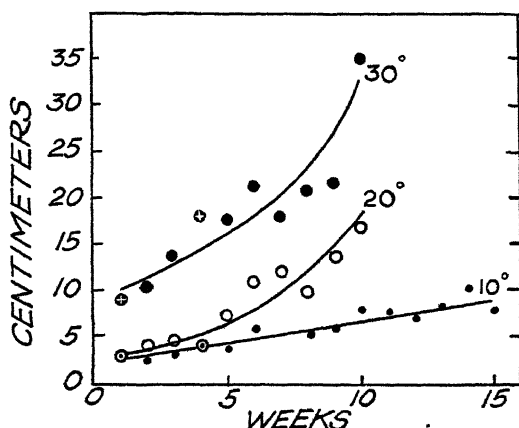


FIG. 4.—Decomposition of sodium chlorate at 10°, 20°, and 30° C. Determined by the height (cm.) attained by barley seedlings when grown in soil to which was added 100 p. p. m. of sodium chlorate

TABLE 5.—Average height attained by barley seedlings planted in soil to which was added 100 p. p. m. of sodium chlorate and kept at different temperatures for increasing lengths of time before planting

Number of weeks interval between application of herbicide and planting of barley seed	Average height of seedlings kept at—			Number of weeks interval between application of herbicide and planting of barley seed	Average height of seedlings kept at—		
	10° C.	20° C.	30° C.		10° C.	20° C.	30° C.
	Cm.	Cm.	Cm.		Cm.	Cm.	Cm.
1.....	3.0	3.0	9.0	9.....	6.0	14.0	22.0
2.....	2.6	4.0	10.4	10.....	8.0	17.0	35.0
3.....	3.0	4.5	14.0	11.....	7.6	-----	-----
4.....	4.0	4.0	18.0	12.....	7.0	-----	-----
5.....	3.6	7.6	17.6	13.....	8.0	-----	-----
6.....	6.0	11.0	21.7	14.....	10.0	-----	-----
7.....	-----	12.0	18.0	15.....	9.0	-----	-----
8.....	5.0	10.0	21.0				

## EXPERIMENTS TO DETERMINE THE EFFECT OF HERBICIDES ON BIOLOGICAL ACTIVITIES IN THE SOIL

The importance of the biological processes occurring in the soil can not be overestimated. Any operation that disturbs the biological balance in a soil will influence its fertility. Partial sterilization of the soil has often increased the fertility, the reason for which has been interpreted in various ways. The application of herbicides in amounts sufficient to eradicate Canada thistle may be suspected to have some influence on the biological activities of the soil. An investigation was made of the effect of the herbicides used in the second field experiment upon the ammonification and nitrification processes, soil protozoa, and earthworms. A short summary of the results is given below.

## EFFECT ON AMMONIFICATION AND NITRIFICATION

Two series of experiments were conducted.

1. The accumulation of ammonia and nitrates was determined in soil samples that were collected on the plots of the second field experiment in the spring of 1927. It was found that the herbicides had no effect on the ammonification process. The accumulation of nitrates was also unaffected by the application of herbicides in the autumn.

2. Definite amounts of herbicides, from 50 to 2,000 p. p. m., were added to soil samples, and the accumulation of ammonia and of nitrates was determined. It was found that sodium chlorate and thiocyanate had no influence on the ammonification process, while cyanide had a retarding effect. The accumulation of nitrates was diminished by all herbicides. Thiocyanate applied at the rate of 500 p. p. m. not only inhibited nitrate accumulation but reduced the amount of nitrate originally present in the soil. The retarding effect of cyanide was less and sodium chlorate had the least influence on the process.

Under field conditions the herbicides should be applied in the autumn. The experiments show that an application at that time has no influence on the ammonification and nitrification processes the following spring.

## EFFECT ON SOIL PROTOZOA

Herbicides were added to soil samples at the rate of 50 to 2,000 p. p. m. After 10 days samples of the cultures were transferred to sterile media and after 10 days of incubation the developing protozoa were studied. The results indicate that sodium chlorate had no influence on the number of protozoa, thiocyanate had a doubtful action, and cyanide had a harmful effect. Later on it was found that protozoa were alive for five days in N/100 solutions of the herbicides. The sodium cyanide solution used in that test was titrated to neutrality. The protozoa seemed very sensitive to the alkaline reaction of an untitrated solution. Thus it seems that at least some of the protozoa are not injured by the herbicides.

## EFFECT ON EARTHWORMS

Earthworms were placed in soil to which herbicides were applied at the rate of 100 to 2,000 p. p. m. After 10 days those surviving were counted. Chlorates produced little or no injury. This result is sup-

ported by observations on experimental plots. The highest amount of thiocyanate killed the worms, and of cyanide even 100 p. p. m. was fatal.

## DISCUSSION

### DISAPPEARANCE OF THE HERBICIDES IN THE SOIL

#### DISAPPEARANCE BY LEACHING

The action of the herbicides under field conditions was of comparatively short duration. Oats grew well on plots that were treated with enough of a herbicide to eradicate Canada thistle if the application was made in the autumn of the previous year. The disappearance of the herbicides may have been due to leaching or decomposition or both.

The curves in Figure 3 show that  $\text{NaClO}_3$  and  $\text{NaCNS}$  penetrate the soil easily. It is clear that the disappearance of these herbicides may, in part at least, be due to leaching. On the contrary,  $\text{NaCN}$  and  $\text{NaHASO}_3$  penetrated the soil but slowly or not at all. Any appreciable leaching of these herbicides is thus not to be expected on heavy soil. In fact, McGeorge (23) found no leaching of arsenic from the surface soil on which plants had been treated with arsenical sprays. Schulz and Thompson (28) found that the greater part of the arsenite applied to a barberry bush had leached out after one year. The soil on which the bush was growing is described as "well drained soil, rich in leaf mold."

#### DISAPPEARANCE BY DECOMPOSITION

##### DECOMPOSITION OF SODIUM CYANIDE

The experiments show that the decomposition of the herbicides varied for each herbicide used.  $\text{NaCN}$  decomposed very rapidly under "natural" conditions, i. e., in soil of optimum moisture content for plant growth. When  $\text{NaCN}$  was added, in amounts of 100 p. p. m. of dry soil, to growing plants it killed them overnight or within a few days. If the application was made at the same time that the barley seed was planted the seedlings grew better in the treated soil than in the untreated controls. Petit (26) also has found that small amounts of  $\text{KCN}$  and other cyanogen compounds increased the yield of some plants in pot cultures. Brenchley (8) and Hawkins (17) found  $\text{HCN}$  or  $\text{KCN}$  to be very toxic to plants grown in solution cultures. Clark (9) found these substances very toxic to fungi.

The fact that  $\text{NaCN}$  decomposes rapidly in soil of optimum moisture content which has a very alkaline reaction suggests that its decomposition is brought about by microorganisms. Results reported by Gardner (14), Robbins (27), and others show that a number of organic substances toxic to higher plants are decomposed by microorganisms.

When cyanides decompose ammonium formate is formed. This compound after proper acidation is probably easily utilized by plants, as is indicated by the beneficial effect of decomposed cyanide. The rapid decomposition of  $\text{NaCN}$  explains why it had no effect on the Canada thistle under field conditions. Its action is reduced still more because it does not easily penetrate the soil.

## DECOMPOSITION OF SODIUM THIOCYANATE

In soil kept at 10° C. an amount of 100 p. p. m. killed barley seedlings from seed planted two weeks after the poison was applied. Barley planted later survived. In soil kept at higher temperatures the poison disappeared earlier. That the decomposition of thiocyanate is not always so rapid is indicated by the action of the thiocyanate in the second field experiment. An application of 250 kgm. NaCNS or more per hectare on March 30 inhibited the growth of oats sown on May 7, or five weeks later. In spite of a rather slow decomposition under wet and cool conditions and rapid penetration through soil, for reasons which are not quite clear, NaCNS failed to eradicate Canada thistles under field conditions.

## DECOMPOSITION OF CHLORATES

Chlorates were found to decompose rather slowly. However, that they decompose is clear. Their disappearance under field conditions is not wholly due to leaching. In soil kept at 30° C.,  $\text{NaClO}_3$  decomposed markedly during 10 weeks. Barley planted 10 weeks after the herbicide was applied grew to full size while that planted earlier died. In soil kept at a lower temperature the decomposition was slower, but noticeable.

The decomposition is probably due to microorganisms. Alvisi and Orabona (2) found that *Penicillium glaucum* Link decomposed  $\text{KClO}_3$  and  $\text{NH}_4\text{ClO}_3$ . Virgili (33) found that chlorates disappeared in 63 days in meat allowed to putrefy. The writer found that species of *Penicillium*, *Aspergillus*, and *Fusarium* grew on top of hay infusions containing N/10  $\text{NaClO}_3$  solution. Numerous bacteria were also observed growing in the solution. Such a solution was found to decompose slowly. For instance, after standing for 60 days with only a very small surface exposed to the air the strength was reduced from N/10 to about N/15. The decomposition thus seems to take place under more or less anaerobic conditions. The decomposition is a reduction by which free oxygen is given off. Its occurrence under anaerobic conditions seems rather natural.

It was observed that the decomposition takes place in soil supersaturated with water. Dunkirk silty clay loam used in the penetration experiments did not give a test for chlorides. However, after a N/10  $\text{NaClO}_2$  solution had leached through for 30 days the leachings gave a good test for chlorides. The chlorate used in the experiment was free from chlorides, so that the chlorides in the penetrating solution appear to be reduced chlorates. The slow decomposition of the chlorates and the ease with which they penetrate a soil explains their destructive effect on the Canada thistle.

## DECOMPOSITION OF ARSENITE

Of the herbicides used in the experiments, sodium cyanide, sodium thiocyanate, and sodium chlorate are compounds of nonpoisonous elements. It is the peculiar combination that makes these compounds toxic. When they are decomposed the constituents are harmless. On the contrary, the toxic element in arsenite is its content of arsenic. It is clear that decomposition of the arsenite does not destroy the poisonous properties of the arsenic. The toxicity may diminish if the



new compound is less soluble, but in solution it is proportional to the arsenic content.

Arsenic combines easily with other elements, so that it is probable that arsenite used as a herbicide decomposes rather rapidly in the soil. As the arsenic is the carrier of the toxic properties it is clear that a soil treated with arsenic can be freed from the poison only by leaching. The experiments show that sodium arsenite penetrates a soil very slowly. Thus a soil treated with appreciable amounts of arsenite remains unfit for plant growth for a long time. Gray (16) reports that plots treated with arsenical spray for the eradication of morning glory were bare of all vegetation for 14 months.

#### THE USE OF HERBICIDES ON ARABLE LAND

The eradication of perennial weeds is an expensive operation. One of the best methods for this purpose is the use of a proper summer fallow. Where labor is inexpensive, the thorough cultivation brought about by the summer fallow is probably the best method for eradication of perennial weeds on arable land. However, the cost of labor may increase and lighter soil may not benefit from the summer fallow tillage as heavy soils have been shown to do. Moreover, fallowing is an operation that is suited only for large areas. Perennial weeds often appear in colonies, and their eradication on smaller areas before they have a chance to spread over the field is desirable.

Chlorates, and especially sodium chlorate, gave the best results of the herbicides tested on Canada thistle in these experiments. It was easily applied as dry salt; it penetrated the soil easily, so that it reached the roots of Canada thistle rapidly; it decomposed comparatively slowly, so that it acted efficiently; it could be applied with little or no interference with crop production; it was found to have but slight influence on the biological activities in the soil.

On very heavy soil the chlorates may be assumed to penetrate too slowly. It was found in the second field experiment that an application of more than 200 kgm. of sodium chlorate per hectare in some cases decreased the yield of oats sown the following spring. The herbicide had not disappeared rapidly enough. The horizontal propagation roots of Canada thistle grow at a depth of 15 to 30 cm. The best time for the application of chlorates was found to be in the autumn after the crop was removed. If the weedy field is to be fall plowed, an application of this herbicide in the furrows by some attachment to the plow would facilitate its action. In this way the herbicide would come in contact with the roots without necessitating its penetration through the first 15 to 20 cm. of soil. Smaller amounts than those used in these experiments may be found sufficient if this scheme is followed.

Chlorates applied in autumn were found to be very efficient for the eradication of Canada thistle. Several other perennial weeds, having less easily affected roots, or rootstocks, are more difficult to eradicate by chlorates. Korsmo (19) found an application of sodium chlorate, 500 kgm. per hectare, insufficient to eradicate quack grass. Fron and Arnal (13) found an application of 2,000 kgm. per hectare necessary for the eradication of *Spartium junceum* L., a leguminous shrub. In both cases the application was made in the spring, which may have diminished the action of the herbicide.

## SUMMARY

The purpose of the experiments here reported was to develop a method for the eradication of Canada thistle, *Cirsium arvense* (L.) Scop., from arable land by the use of herbicides. The additional effects of the herbicides upon the soil were also determined.

Sodium chlorate,  $\text{NaClO}_3$ ; potassium chlorate,  $\text{KClO}_3$ ; sodium thiocyanate,  $\text{NaCNS}$ ; sodium cyanide,  $\text{NaCN}$ ; and sodium arsenite,  $\text{NaHAsO}_3$  were the herbicides used.

An application of 200 kgm. per hectare of sodium chlorate (or 250 kgm. potassium chlorate) per hectare as dry salt on the ground late in the autumn killed the roots of Canada thistle during the winter. An application early in the spring was less effective. Other herbicides used had practically no effect on the Canada thistle under field conditions.

The effectiveness of chlorates is due to their rapid penetration through soil and their slow decomposition, especially at low temperatures. Sodium thiocyanate, and to a greater extent sodium cyanide, decomposed so rapidly in the soil that no harm was done to Canada thistle under field conditions. Sodium cyanide did not seem to penetrate the soil under field conditions. Sodium arsenite was ineffective against Canada thistle because it penetrated the soil very slowly. A special apparatus was constructed for determining the rate at which the herbicides penetrated the soil.

An application of herbicides in the autumn had no influence on the ammonification and nitrification processes in the soil the following spring.

An application of 200 kgm. per hectare of sodium chlorate in late autumn killed the Canada thistles and did not injure the oats that were sown on the plots the following spring.

## LITERATURE CITED

- (1) ANONYMOUS.  
1901. DESTRUCTION OF PRICKLY PEAR. Queensland Agr. Jour. 9: 460-462.
- (2) ALVISI, U., and ORABONA, M.  
1912. SUL COMPORTAMENTO DI PERCLORATI E CLORATI, NITRATI E NITRITI IN ALCUNI ESPERIMENTI DI CHIMICA BIOLOGICA E SUL POTERE RIDUCENTE DE' TUBERCOLI RADICALI DELLE LEGUMINOSE (ES. VICIA FABA.) Gaz. Chim. Ital. 42 (1): 565-575.
- (3) ÅSLANDER, A.  
1925. NÅGRA RESULTAT AV OGRÄSFÖRSÖK SOMMAREN 1924. [SOME RESULTS OF EXPERIMENTS OF WEED ERADICATION DURING THE SUMMER OF 1924.] Landtmannen 8: 300-301.
- (4) ———  
1926. ERFARENHETER FRÅN OGRÄSFÖRSÖK SOMMAREN 1925. [EXPERIENCES OF WEED ERADICATION DURING THE SUMMER OF 1925.] Landtmannen 9: 353-357, illus.
- (5) ———  
1926. CHLORATES AS PLANT POISONS. (A PRELIMINARY REPORT.) Jour. Amer. Soc. Agron. 18: 1101-1102.
- (6) ———  
1927. SULPHURIC ACID AS A WEED SPRAY. Jour. Agr. Research 34: 1065-1091, illus.
- (7) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.

- (8) BRENCHELEY, W. E.  
1917. ORGANIC PLANT POISONS. I. HYDROCYANIC ACID. *Ann. Bot.* [London] 31: [447]-456, illus.
- (9) CLARK, J. F.  
1899. ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI. *Bot. Gaz.* 28: 289-327, 378-404, illus.
- (10) DETMERS, F.  
1927. CANADA THISTLE, *CIRSIMUM ARVENSE* Tourn. FIELD THISTLE, CREEPING THISTLE. *Ohio Agr. Expt. Sta. Bul.* 414, 45 p., illus.
- (11) FEILITZEN, H. VON  
1925. NATRIUMKLORAT. ETT UTMÄRKET MEDEL ATT HÅLLA TRÄDGÅRDSGANGAR, GÅRDS—OCH TENNISPLANER M. M. FRIA FRÅN OGRÄS. [SODIUM CHLORATE; AN EXCELLENT REMEDY AGAINST WEEDS ON TENNIS COURTS, ETC.] *Svenskt Land* 9: 267-269.
- (12) ———  
1925. NATRIUMKLORAT. YTTERLIGARE NÅGRA FÖRSÖK DÄRMED. [SODIUM CHLORATE; SOME FURTHER TRIALS.] *Svenskt Land* 9: 342-343.
- (13) FRON, G., and ARNAL, A.  
1927. ESSAIS DE DESTRUCTION DE GENÊTS PAR LE CHLORATE DE SOUDE. *Compt. Rend. Acad. Agr. France* 13: 215-217. (Discussion, p. 217-219.)
- (14) GARDNER, W. A.  
1926. THE DECOMPOSITION OF TOXINS BY SOIL ORGANISMS. *Ala. Agr. Expt. Sta. Bul.* 225, 38 p.
- (15) GRAY, G. P.  
1917. SPRAYING FOR THE CONTROL OF WILD MORNING-GLORY WITHIN THE FOG BELT. *Calif. Agr. Expt. Sta. Circ.* 168, 7 p.
- (16) ———  
1919. TESTS OF CHEMICAL MEANS FOR THE CONTROL OF WEEDS. REPORT OF PROGRESS. *Calif. Univ. Pubs., Agr. Sci.* 4: [67]-97, illus.
- (17) HAWKINS, R. S.  
1925. HYDROCYANIC ACID AS A TOXIC AGENT TO PLANT GROWTH. *Jour. Amer. Soc. Agron.* 17: 169-171, illus.
- (18) KORSMO, E.  
[1925]. UGRESS I NUTIDENS JORDBRUK. [WEEDS IN PRESENT DAY AGRICULTURE.] 694 p., illus. Oslo.
- (19) ———  
1927. NATRIUMKLORAT ( $\text{NaClO}_3$ ) SOM UGRÆSDRÆPENDE MIDDEL. [SODIUM CHLORATE AS A REMEDY AGAINST WEEDS.] *Norsk Landmandsblad* 46: 146-148.
- (20) LATSHAW, W. L., and ZAHNLEY, J. W.  
1927. EXPERIMENTS WITH SODIUM CHLORATE AND OTHER CHEMICALS AS HERBICIDES FOR FIELD BINDWEED. *Jour. Agr. Research* 35: 757-767, illus.
- (21) LOYER.  
1923. EMPLOI DES CHLORATES POUR LA DESTRUCTION DES HERBES DANS LES CÉRÉALES DE PRINTEMPS. *Compt. Rend. Acad. Agr. France* 9: 957-960.
- (22) LUND, S., and ROSTRUP, E.  
1901. MARKTIDSELEN, *CIRSIMUM ARVENSE*. EN MONOGRAFI. [CANADA THISTLE, *CIRSIMUM ARVENSE*. A MONOGRAPH.] K. Danske Vidensk. Selsk. Skr. Naturv. og Math. Afd. (6) 10: [149]-313, illus. [French résumé, p. 301-313.]
- (23) McGEORGE, W. T.  
1915. THE EFFECT OF ARSENITE OF SODA ON THE SOIL. *Hawaii Agr. Expt. Sta. Press Bul.* 50, 16 p., illus.
- (24) MUNN, M. T.  
1919. SPRAYING LAWNS WITH IRON SULFATE TO ERADICATE DANDELIONS. *N. Y. State Agr. Expt. Sta. Bul.* 466, p. 21-59, illus.
- (25) PACZOSKI, J.  
1916. A BIOLOGICAL PECULIARITY OF *CIRSIMUM ARVENSE* Scop. *Trudy Biūro Prikl. Bot. (Bul. Appl. Bot.)* 9: 1-16, illus. [In Russian. English résumé, p. 14-16.]
- (26) PETIT, A.  
1926. INFLUENCE SUR LA VÉGÉTATION DE QUELQUES COMPOSÉS CYANO-GENÉS. *Jour. Agr. Prat. (n. s.)* 90: 490-491.

- (27) ROBBINS, W. J.  
1917. THE CAUSE OF THE DISAPPEARANCE OF CUMARIN, VANILLIN, PYRIDINE AND QUINOLINE IN THE SOIL. *Ala. Agr. Expt. Sta. Bul.* 195, p. 49-64, illus. (Tech. Bul. 2.)
- (28) SCHULZ, E. R., and THOMPSON, N. F.  
1925. SOME EFFECTS OF SODIUM ARSENITE WHEN USED TO KILL THE COMMON BARBERRY. *U. S. Dept. Agr. Bul.* 1316, 19 p., illus.
- (29) STATENS FORSOGSVIRKSOMHED I PLANTEKULTUR.  
1925. BEKAEMPELSE AF UKRUDT MED OVERBRUSNING MED FORSKELLIGE VAEDSKER. [ERADICATION OF WEEDS BY SPRAYS.] *Ugeskr. Landm.* 70: 265-267.
- (30) STEWART, G., and PITTMAN, D. W.  
1924. STUDIES IN THE ERADICATION OF WILD MORNING-GLORY. *Jour. Amer. Soc. Agron.* 16: 506-518.
- (31) THOMPSON, N. F., and ROBBINS, W. W.  
1926. METHODS OF ERADICATING THE COMMON BARBERRY (*BERBERIS VULGARIS* L.). *U. S. Dept. Agr. Bul.* 1451, 46 p., illus.
- (32) TREADWELL, F. P.  
1911-13. ANALYTICAL CHEMISTRY. Translated from 5th German ed. by W. T. Hall. Ed. 3, rev. and enl., 2 v., illus. New York and London.
- (33) VIRGILI, J. F.  
1909. RECHERCHE TOXICOLOGIQUE DES CHLORATES. *Ann. Chim. Analyt* 14: 289-294.

# DOWNY MILDEW (*SCLEROSPORA GRAMINICOLA*) ON EVERGLADE MILLET IN FLORIDA<sup>1</sup>

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## INTRODUCTION

Among the downy mildews or Peronosporaceae, the genus *Sclerospora* has attracted increasing attention on account of its destructive parasitism of various valuable gramineous crops. Of this genus, the species *Sclerospora graminicola* (Sacc.) Schroet. is the most widely distributed, occurring in northern temperate portions of the United States and Europe and in both temperate and tropical regions of Asia, and causing in the aggregate considerable injury to millet and related crops. Yet the present knowledge of this fungus in many respects is scanty, and additional information as to its occurrence in new localities or on additional hosts, its severity, persistence, and spread under different conditions, is necessarily of interest from both the agricultural and mycologic points of view.

Therefore, when this species, for the first time in this State and on this host, was found by the junior writer in November, 1922, on Everglade millet at Vero Beach, Fla., and when during the following year it was encountered also in other widely separated parts of the State, it seemed important to investigate the destructive activity of the parasite on this new host under subtropical conditions and to appraise the possibility of its becoming a menace to Florida's agriculture. The results of this investigation are presented in the following paper.

## THE DISEASE

### OCCURRENCE IN FLORIDA

Ever since the first discovery of the fungus in Florida an intermittent but extensive search has been made to determine on what other hosts and in what other parts of the State it might occur. Up to the present, however, only the one species of host has been found to be infected under natural conditions. This grass, the Everglade millet,<sup>3</sup> *Chaetochloa magna* (Griseb.) Scribner (*Setaria magna*, Griseb.), is very common throughout the State in low marshy ground

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<sup>2</sup> The senior writer wishes to express his thanks to the Office of Cereal Crops and Diseases for supporting his share of these investigations in Florida in June, 1924, while on leave from Harvard University, and to Hugh M. Matheson, of Miami, Fla., for courtesies which facilitated the intensive study of the mildew. He wishes also to acknowledge his indebtedness for aid from the Milton Research Fund, of Harvard University, which expedited the long-delayed preparation of the manuscript and plates by relieving him of some of the demands of academic work.

<sup>3</sup> The writers take this opportunity of thanking Mrs. Agnes Chase for her kindness in verifying the identification of the host.

on muck or peat soils, either quite outside of cultivation or as a weed in poorly cultivated fields. In the monograph of Scribner and Merrill (*14*, p. 21)<sup>4</sup> the grass is described as "a coarse, stout, erect perennial (?) 10 to 36 dm. high, with cylindrical culms 0.5 to 2 cm. thick at the base, linear-lanceolate leaves and dense, cylindrical panicles 1.5 to 3 dm. long, \* \* \* from Delaware to Florida, Louisiana, and western Texas." Of its perennial character there may be doubt, as indicated by the question mark, in the northern

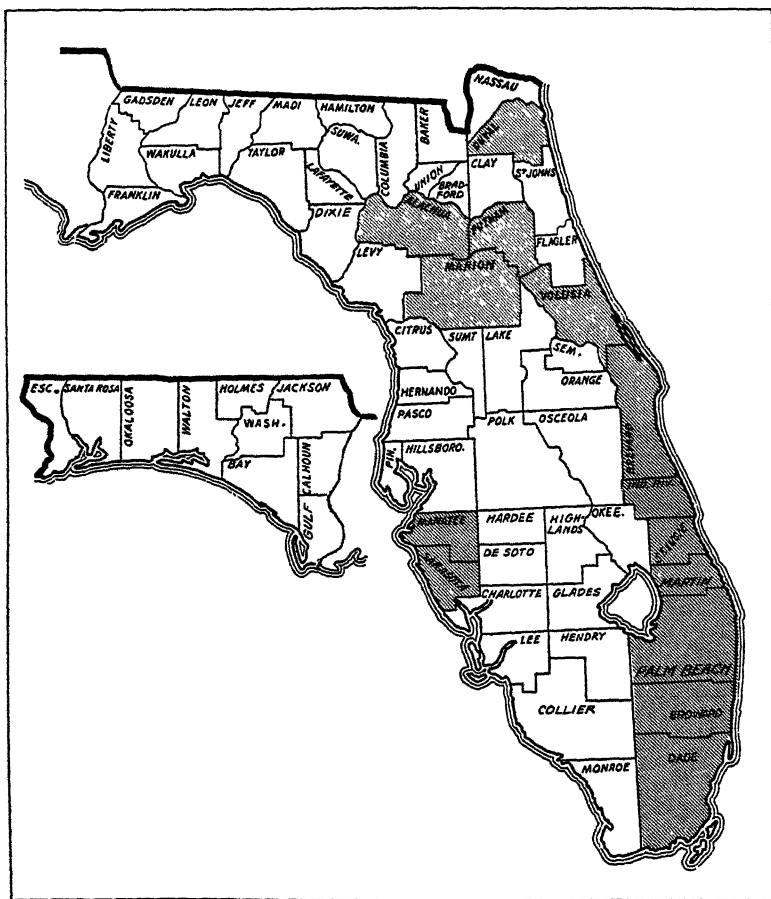


FIG. 1.—Sketch map of Florida. Shaded areas show counties in which *Sclerospora graminicola* has been collected on Everglade millet

limits of its range; but in Florida, at least, it is indeed perennial, forming persistent, heavy clumps that continually put forth new growths.

In Florida, as noted by the writers in a preliminary report (*22*), the fungus invariably has been found wherever this grass was encountered, the distribution of the parasite and of the host apparently being coincident. So far they have been collected from various parts

<sup>4</sup> Reference is made by number (*italic*) to "Literature cited," p. 962.

of the following counties: Dade, Broward, Palm Beach, Martin, St. Lucie, Indian River, Brevard, Volusia, Duval, Putnam, Alachua, Marion, Manatee, and Sarasota. As is obvious from the accompanying map (fig. 1), these localities cover representative regions of the whole State from the more northern portion that resembles adjacent territory of sister States to the subtropical peninsula which is peculiar to Florida.

This occurrence of *Sclerospora graminicola* on *Chaetochloa magna* in Florida is an interesting addition to our knowledge of the host range and the geographic distribution of the fungus. Since the species was described first by Schroeter (13) in 1879 from Baden, Germany, on *Setaria* (*Chaetochloa*) *viridis* Beauv., it has been reported most commonly throughout Europe on this host from various localities in Italy, France, Germany, Czechoslovakia, and Russia, while on the two closely related wild grasses *C. glauca* (L.) Scribn. and *C. verticillata* (L.) Scribn. it has been found also with a very similar distribution. On the cultivated Italian millet, *C. italica* (L.) Scribn., it has been reported not only throughout Europe but also from points in India, China, and Japan. On *Pennisetum typhoideum* Rich. it has been recorded from German East Africa and from two widely separated points in India. It is of interest that all of these hosts are in the tribe Paniceae of the family Gramineae. As to the occurrence of this fungus on hosts outside of this tribe, there is the somewhat doubtful record on sugar cane in England (8) and the record of its occurrence in the oogonial stage in tassels of maize in South America (16)—a case in which it seems more probable that the fungus was *Sclerospora macrospora*. The variety *S. graminicola* var. *andropogonis-sorgi* of Kulkarni (?) on sorghum, as has been pointed out also in a previous paper (21), is sufficiently distinct to warrant its being established as a separate species.

In the United States, *Sclerospora graminicola*, since it was reported first by Farlow in 1884 (4) on the green foxtail grass, *Chaetochloa viridis*, from La Crosse, Wis., has been recorded chiefly on that host, although there have been a few records of its occurrence on the closely related smooth foxtail grass, *C. glauca* (L.) Scribn., and several reports of it on the cultivated Italian millet, *C. italica* (L.) Scribn., and on the German or Hungarian variety, *C. italica* var. *germanica* (Mill.) Scribn. These records are chiefly from the northernmost line of States, New York, Michigan, Wisconsin, Minnesota, and North Dakota, with some records also from Iowa, Illinois, and South Dakota, while, in so far as the writers have been able to determine, the most southern records hitherto have been from Kansas and New Mexico.

To these previous records this new note of the occurrence of *Sclerospora graminicola* on *Chaetochloa magna* in Florida is an addition of considerable significance. Hitherto the fungus has been found in the United States, not on native hosts but on hosts which have been introduced from Europe, even the widespread and well-established *C. viridis* being of European origin. This has suggested strongly that the parasite, as it was found only on these hosts, was of European origin as well. The widespread distribution in Florida, however, on such an indigenous grass under wild conditions seems to justify the assumption that the fungus also may be native to the

southeastern part of the United States. The other possibility, that at some time in the past the fungus may have escaped from cultivated millet and become established on this wild host, seems opposed by the fact that millet has been and is very little cultivated in the State, and that the fungus on the wild host under natural conditions is so generally distributed and apparently so long established in wild localities remote from cultivation.

Indeed, so regularly is the fungus associated with *Chaetochloa magna* in Florida that it seems highly probable it will be found on this host elsewhere. The geographic range of the grass in the United States is not limited to Florida, but extends along the southern Atlantic seaboard from Delaware to Florida and along the Gulf States from Louisiana to western Texas, while outside of the United States the grass is known in Bermuda, the West Indies, and Central America. From the fact that the occurrence of this downy mildew parasite in Florida is so precisely coincident with the occurrence of the grass host, the writers feel that it is justifiable perhaps to expect that this same coincident occurrence will be found in other parts of the range of the grass.

#### SYMPTOMS AND EFFECT

The symptoms which Everglade millet displays when infected by *Sclerospora graminicola* are relatively easily recognized. Infection most commonly is systemic, and the symptoms agree in general with those characterizing the attack of this fungus on other hosts. Also, however, local infection restricted to scattered spots on the leaves was found to occur—a less obvious type of infection which, as far as the writers are aware, hitherto has not been recorded for this fungus.

#### SYSTEMIC INFECTIONS

Systemic infection shows itself chiefly in the development of pallid yellowish markings in the dark-green tissue of the leaf. (Pl. 1, A, D, and E.) On the lowest (i. e., oldest, earliest unfolded) leaf thus marked, the pallid area is restricted to the base of the leaf; but on each successive leaf unfolding thereafter the areas are more extensive, running out in irregular, jagged extensions, progressively farther and farther toward the tip, the latest leaves being completely yellowish white throughout. The characteristic markings on successive leaves of a typically infected plant are shown in the accompanying diagram (fig. 2), which was prepared by tracing carefully with a sharp stylus through the tissue of spread-out leaves. These markings may appear very early in the life of the plant or when it is half grown or older, the first (lowest) leaf to show these areas being any leaf from the third or fourth to the eighth or ninth. Seedlings that are severely attacked are readily distinguished from their healthy fellows on casual examination, even when only a few inches tall, by their pallid, marked appearance. (Pl. 1, A.) Young plants that are growing rapidly may be a foot or two tall before the fungus invades the later unfolding leaves and they become symptomatically pallid.

In general these symptomatic markings of the affected plants resemble those characteristically produced by other members of the





FIG. 2.—Characteristic markings of systemic infection. Diagrams showing the typical configuration and extent of the etiolated conidiophore-bearing areas on successive leaves of a *Chaetochloa magna* plant systemically infected by *Sclerospora graminicola*. Drawn from life-sized tracings, scale in inches. The formation of resting spores (oogonia containing oospores) already has begun in the tissue of the older leaves in the stippled spots at  $O_1$  and  $O_2$ . The affected, etiolated area (white) is increasingly greater and the unaffected area (black) increasingly smaller on successive leaves. The actual and the proportionate extent of these areas was as follows:

Item	Leaf No.										Total
	11	10	9	8	7	6	5	4	3	2 and 1 withered	
Leaf area.....sq. cm.	35.2	69.78	89.76	107.60	123.86	134.9	87.38	61.80	23.1	0	733.38
Conidiophore-bearing area.....do.	35.2	69.78	89.76	62.88	46.86	13.5	0	0	0	0	317.98
.....(per cent.)	100	100	100	58.44	37.83	10	0	0	0	0	43.36

genus *Sclerospora* that have been studied intensively. The similarity between the pattern and configuration of these areas, shown here in Figure 2, and those figured in earlier papers (19, *pl. B*; 20, *fig. 1*) for the Philippine mildews of maize, is quite striking. The actual area of these symptomatic markings, being proportionate to that of the large coarse leaves of the Everglade millet, is quite extensive, even as large as 317.98 sq. cm. in the case of some medium-sized systemically infected plants. (See Table 1.) As the production of conidiophores from the mycelium within the host takes place on these areas, the extent of this production also can be gauged and compared. In such a comparison (Table 1) the conidiophore-bearing area of leaves of Everglade millet systemically infected with *Sclerospora graminicola*, although this production takes place on only one surface, is found to compare very well with similar areas on maize plants systemically infected with *S. philippinensis*, even though in maize the production of conidia customarily is on both sides of the leaf. In this comparison the areas of the leaf sheaths as formerly recorded (20, *Table 2*) are omitted to make the comparison more just, for little if any production of spores takes place on the leaf sheaths in Everglade millet.

TABLE 1.—Conidiophore-bearing areas on representative host plants infected by species of *Sclerospora*

Comparison of the extent of sporulating areas on (1) *Chaetochloa magna* plants, showing inconspicuous local-spotting type of infection with *Sclerospora graminicola*; (2) *C. magna* plants showing the conspicuous systemic type of infection with *S. graminicola*; and (3) *Zea mays* plants of representative sizes of two varieties showing systemic infection with *S. philippinensis* previously recorded by Weston (20, *Table 2*)

Items compared, variety, and plant number	Number of leaves	Approximate height of plant (cm.)	Total area of leaves (sq. cm.)	Conidiophore-bearing area	
				Sq. cm.	Per cent
Local infections on <i>C. magna</i> by <i>S. graminicola</i> :					
No. 1.....	8+2 withered.....	-----	<sup>a</sup> 925.60	<sup>a</sup> 3,665	3.90
Systemic infections on <i>C. magna</i> by <i>S. graminicola</i> :					
No. 2.....	9+2 withered.....	-----	<sup>a</sup> 733.38	<sup>a</sup> 31,798	43.36
Systemic infections on <i>Zea mays</i> by <i>S. philippinensis</i> :					
Native Yellow Flint, No. 5.....	9.....	91	<sup>b</sup> 1,819.02	<sup>b</sup> 35,170	19.33
Native Yellow Flint, No. 10.....	12+ tassel.....	192	<sup>b</sup> 5,127.90	<sup>b</sup> 262,920	51.27
Mexican June, No. 11.....	9+ tassel.....	126	<sup>b</sup> 1,502.78	<sup>b</sup> 134,655	89.60
Mexican June, No. 16.....	.....do.....	154	<sup>b</sup> 3,656.79	<sup>b</sup> 128,580	35.16

<sup>a</sup> Lower surfaces only.

<sup>b</sup> Both surfaces.

On these pallid areas there develops during favorable dewy nights a downy growth of conidiophores and conidia (zoosporangia), the presence of which, of course, is an additional and conclusive symptom.

When plants show the disease while still small seedlings, presumably as a result of very early infection, they may be killed in a relatively few weeks or may struggle on for some time without developing any additional destructive symptoms.

When plants, presumably after much later infection, do not show the disease until partly grown, the effects of the parasite run on in a further symptomatic course. Such plants as they mature usually become distinctly stunted in comparison with the healthy ones (*pl. 2*,

A), and in some cases also put out an abnormal abundance of suckers that give a bunchy, rosetted appearance to the clumps (pl. 2, B). As a rule, if one shoot from a clump shows systemic infection, all those developing subsequently will do likewise. (Pl. 1, B, C.)

On the Everglade millet, as on its other hosts, the fungus runs through the two typical reproductive phases of its life history. On the pallid areas occurs first the formation of the conidial stage, which continues on the leaves, already unfolded, during favorable dewy nights and begins anew on the pale portions of the leaves that develop subsequently. In time, however, as the host matures, the production of conidiophores on the surface of the leaves lessens and gradually gives way to the formation of oogonia from the mycelium within the host tissue. The production of oogonia usually begins in the older, lower leaves and goes on progressively upward in leaf after leaf until established last in the topmost, youngest leaves, which may continue to support conidiophore production even until the terminal inflorescence is developed and long after the lower leaves are riddled with oogonia.

Of this oogonial phase of the disease certain additional symptoms are characteristic. When the intramatrical hyphae begin to form oogonia, the infected areas of the host tissue in which they are located change from the pallid, yellowish color that has characterized them to a somewhat reddish brown. In time the leaf tissue between the bundles disintegrates (pl. 2, E, F), the bundles persisting as the leaves become shredded, until only a tangle of frayed fibers is left (pl. 2, C). Usually, also, the inflorescence or head of the grass is not normally developed, but is decidedly stunted and virescent or otherwise teratologically malformed (pl. 2, D), much as are those so carefully described by Butler (2) in the case of *Pennisetum typhoideum* when attacked by this fungus in India. As a rule such heads are sterile, and the florets, instead of producing seed, grow into small clusters of bracts, the palea and lemma particularly tending to develop abnormally into straplike or leaflike growths. These abnormal structures are permeated also by the mycelium of the parasite, and they may for a time support production of conidiophores. Ultimately they fray out into frazzled fibrous tangles as the interfascicular tissue disintegrates with the maturing of the oogonia.

#### LOCAL INFECTIONS

In addition to the usual systemic type of infection just considered, there occurs on the Everglade millet, as has been mentioned above, a very different, local type of infection. In this type the infection of the host is limited to restricted spots on the leaves. While the cases of systemic infection immediately attract attention because of the conspicuous symptoms of the disease—the obvious, pallid markings, stunted growth, and malformed inflorescences—the locally infected plants show no striking symptoms and in consequence are likely to escape notice. They are relatively numerous, however, many fields of the grass showing hardly a single apparently healthy plant that on close examination does not have at least a few scattered spots of local *Sclerospora* infection on its leaves. In one

typical field near Miami, along Cocoplum Beach Road in low land formerly under cultivation but grown up to a rank growth of Everglade millet when examined, 202 plants chosen at random were inspected carefully. Only 2 were found to be quite healthy without any signs of *Sclerospora* infection whatever, while 149 showed more or less abundant spotting of local infection, and 51 displayed obvious symptoms of systemic attack.

Although no extensive counts were made, some attention was paid to the relative frequency of occurrence of both types of infection around Miami, and the proportion given above was found to be a fairly typical one. A very large proportion of the tall vigorous plants that on casual examination appeared to be entirely free from *Sclerospora* were found on closer inspection to have at least a few local infection spots, chiefly on the upper, younger leaves. In some cases both types of infection were found on the same plant, the plants systemically attacked showing also scattered, locally infected spots either near the base of the plant on the wholly green lower leaves below those that showed the progressively pallid markings typical of systemic infection, or even on these leaves themselves, although only on their green unaffected tips. In no cases were local infection spots found in the pallid areas of leaves already invaded by the mycelium of systemic infection.

From the healthy, normal green, uninvaded tissue of the leaves the invaded locally infected spots were distinctly defined by being somewhat sunken, of a rather irregularly roughened surface texture, and of a buff to dull reddish to very dark-brown color.

In shape and size these spots of local infection varied from irregular, somewhat rectangular, areas of only a few square millimeters in extent to extensive linear streaks running lengthwise of the leaf. (See fig. 3.) In general, rather sharply bounded laterally by the leaf veins (though sometimes irregularly jagged), and varying from 1 to as much as 10 mm. in width, they are relatively elongate, ranging from one-fifth to perhaps even 10 cm. in length.

#### EXPLANATORY LEGEND FOR PLATE 1

A.—Young plant of Everglade millet showing systemic infection with *Sclerospora graminicola*. Beginning with the fifth leaf, the characteristic elongate, pallid markings are clearly evident, and on them the production of the downy mildew is begun. About one-fourth natural size. (Photographed at Coconut Grove, Fla., June 22, 1924, by G. F. Weber.)

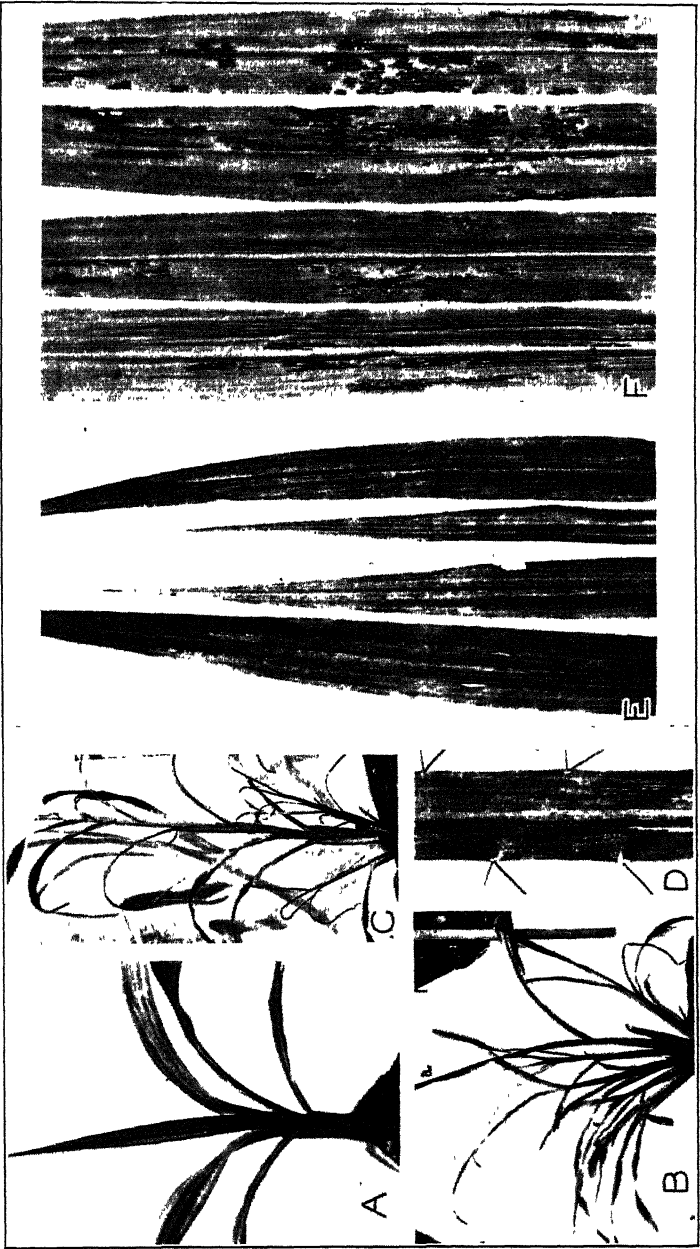
B.—A young Everglade millet plant of six shoots in which infection by the downy mildew presumably occurred after the last largest shoot (a) already was developed, as this one is healthy while the other five younger, smaller, more recently developed shoots all show peculiar systemic general infection by the downy mildew. (Photographed along the Cocoplum Beach Road in June, 1924, by W. H. Weston, jr.)

C.—A vigorous yet badly infected Everglade millet plant, the largest 3-foot shoot of which shows characteristic systemic infection of the downy mildew and is still supporting conidiophore production on its leaves, while in the two smaller and stunted systemic-infected shoots at its base the production of conidiophores has ceased and already the formation of the resting spores in the oogonia has begun in the curled and drying leaf tissue. (Photographed along the Cocoplum Beach Road in June, 1924, by W. H. Weston, jr.)

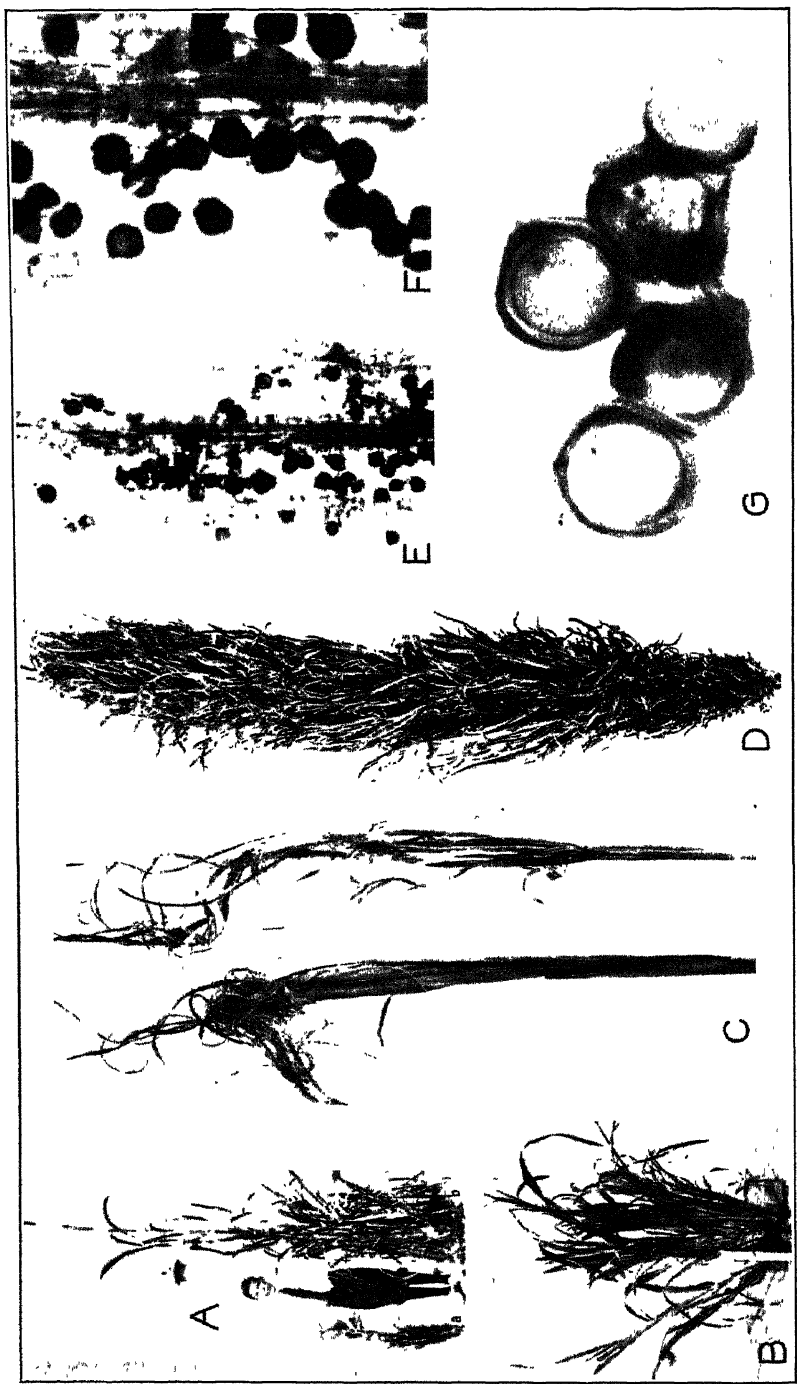
D.—Portion of the upper surface of the eighth leaf of a systemically infected plant with fairly conspicuous symptomatic etiolated conidiophore-bearing areas.  $\times \frac{1}{2}$ . (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

E.—Successive leaves of Everglade millet showing the elongate pallid markings of systemic infection by the downy mildew with conidiophore production still taking place on them. (Photographed at Florahome, Fla., April, 1924, by G. F. Weber.)

F.—Successive leaves of large Everglade millet plant showing the characteristic irregular spotting of the local type of infection with the downy mildew. (Photographed at Florahome, Fla., April, 1924, by G. F. Weber.)



(For explanatory legend, see page 942)



(For explanatory legend, see page 943)

The spots occurred not only separately and scattered but also coalesced into larger areas of more or less irregular outline. Their distribution on the leaves showed no definite pattern or arrangement but was decidedly irregular.

The characteristic appearance of these areas of local infection is well brought out in Plate 1, F. Their size, shape, and arrangement also are shown in that illustration and in the outline diagram of Figure 3, which was traced from a typically representative, locally infected plant. The actual area of these locally infected spots is relatively small, occupying in a typical case such as the one tabulated, only 36.65 sq. cm., or a little less than 4 per cent of the total leaf area. Obviously (see Table 1), this is relatively small, also, compared to the much greater extent of the area that is etiolated and produces conidia in the case of the systemic infections.

The production of *Sclerospora conidiophores* took place almost exclusively from the undersurfaces of these spots, and under favorable circumstances the fungous growth could be seen as a sparse down recognizable with a hand lens. The conidiophore production was always rather scanty in density and abundance compared to that on the etiolated tissue of systemically infected plants. As the plants aged, the locally infected tissue merely ceased to produce conidia and, so far as was observed, dried up without ever developing oogonia or fraying out with the disintegration of interfascicular tissue that accompanies this process.

On the whole, there was nothing outstandingly distinctive about the symptomatic markings of this local type of infection, the spots to the casual glance resembling those caused by *Helminthosporium*, for example. The facts that it is not outstandingly distinctive or conspicuous and that it at first escaped their attention in fields where it was very common lead the writers to suspect that, although

#### EXPLANATORY LEGEND FOR PLATE 2

A.—A comparison of a normal-sized, vigorous, fruiting, healthy clump of Everglade millet (b) with the characteristically stunted, poorly developed, sterile plant heavily systemically infected with *Sclerospora graminicola* (a). The boy holding the plant is about 4½ feet tall. (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

B.—A stunted plant of Everglade millet with the dense bunchy growth which results from early and long-standing infection with downy mildew, now given over almost wholly to the production of oogonia in its tissues. (Photographed at Coconut Grove, Fla., June, 1924, by W. H. Weston, jr.)

C.—Characteristically shredded fibrous tops of plants which have been killed by the downy mildew and now in the late stages of the disease are given over to the production of oogonia, with a disintegration of the tissue between the bundles and the consequent fibrous tangles of the dry bundles from which the resting spores are constantly scattering. About two-thirds natural size. (Photographed at Belle Glade, Fla., July, 1924, by G. F. Weber.)

D.—The head or inflorescence of the Everglade millet plant badly infected by the downy mildew, showing the abnormal development of elongate, straplike bracts in the place of the normal parts of the flowers, so that the whole head is sterile and virescently malformed as a result. The tissue is full of oogonia but has not as yet shredded out to set the spores free. (Photographed at Pomona, Fla., May 28, 1924, by G. F. Weber.)

E.—Tissue of a portion of the leaf of Everglade millet showing the location of the oogonia in the disintegrating tissue between the fibrovascular bundles.  $\times 75$ . (Photomicrograph by S. A. Howes.)

F.—A more highly magnified view of the same sort of leaf tissue which has begun to shred out in the late stages of the disease, showing the position of the resting spores in their relation to the bundles.  $\times 125$ . (Photomicrograph by S. A. Howes.)

G.—Resting spores (oospores) surrounded by the closely enveloping, thickened, irregular, ogonial wall; cleared and photographed in lacto-phenol solution to show the structure of the oospores and their relation to the enveloping oogonia.  $\times 350$ . (Photomicrograph by S. A. Howes.)

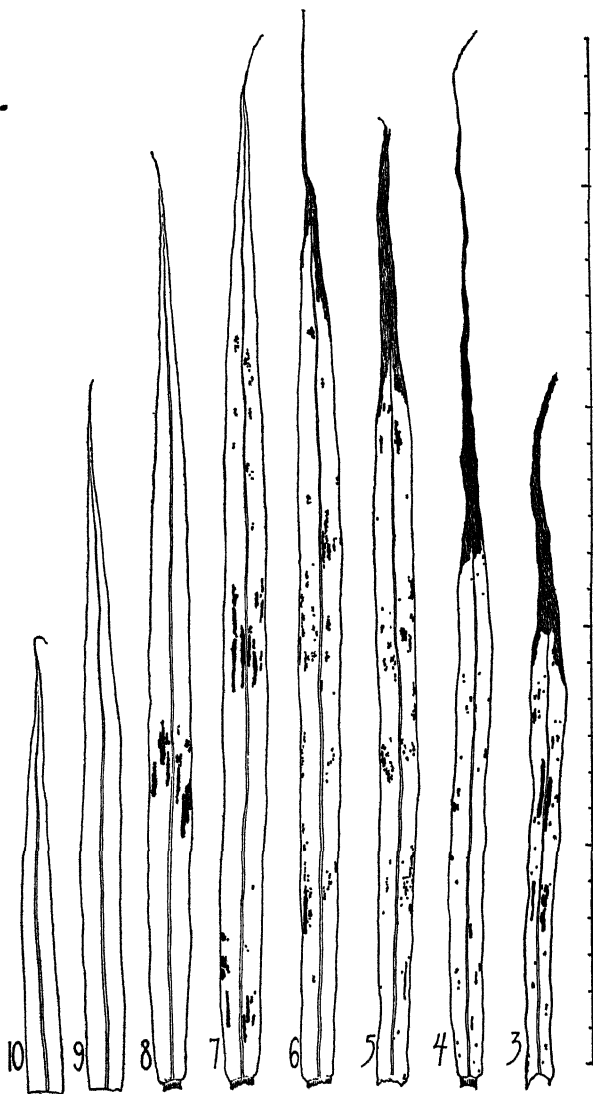


FIG. 3.—Characteristic markings of local spot infection. Diagrams showing the typical shape, size, and distribution of the reddish-brown discolored areas bearing conidiophores on successive leaves of a *Chaetochloa magna* plant locally infected by *Sclerospora graminicola*. As is customary with this type of infection, no formation of resting oospores occurred. Drawn from life-sized tracings, scale in inches, the spots distinguished by shading from the unaffected leaf tissue (white). The irregularity in the distribution of the spots and in the area they occupy on different leaves is distinctly in contrast to the situation in systemic infection. The actual and the relative extent of these areas was as follows:

Item	Leaf No.									Total
	10	9	8	7	6	5	4	3	2 and 1 withered	
Leaf area.....sq. cm.....	49.96	93.22	133.4	167.4	155.06	141.08	105.78	79.7	0	925.60
Conidiophore-bearing area.....do.....	0	0	4.97	9.19	6.82	7.09	2.48	6.1	0	36.65
.....per cent.....	0	0	3.7	5.5	4.4	5.0	2.3	7.6	0	3.96



apparently not yet reported, this local infection by *Sclerospora graminicola* perhaps occurs on other hosts and merely has remained unnoticed. In any case, its occurrence on Everglade millet is of considerable interest, first, because of its possible significance, and second, because of its possible importance in the distribution of the disease. That *Sclerospora graminicola* should so frequently manifest itself in this local type of infection, so different from the customary systemic one, suggests either that a different strain or physiologic race is responsible or that the same strain which usually accomplishes systemic infection may produce very dissimilar effects on the host under different conditions. If, indeed, a distinct strain is concerned, one would expect to find the physiological difference in the activity of the strain accompanied by recognizable though perhaps slight distinctions in such morphological criteria as the size of the conidia. While a slight difference was indeed found in size of the conidia from local infections in comparison to that shown in Table 2, it was not regarded by the writers as sufficiently conclusive evidence of a distinct strain; (1) because it was very slight (only  $2\ \mu$  longer and  $2\ \mu$  broader); (2) because it was based on so few measurements (only 50 spores, from one plant, during one night in the scanty opportunity for night work at Miami in June, 1924); and (3) because it was not accompanied by any difference in shape (the most common ratio of length over width remaining the same, 1.25 to 1.34) or by any difference in the essential size or structure of the conidiophores. Further measurements should be made to settle this point. With the evidence at hand, however, it seems much more probable to the writers that the same strain of *Sclerospora graminicola*, acting under different conditions, produced both local and systemic infection.

The impression gained by extensive field observations is that the point of infection and the age of the host are instrumental in determining the type of infection that will result. Apparently, if inoculation takes place by spores falling into the unfolding, dew-containing terminal bud, the resulting attack will be systematic, even if the plant is relatively well grown. Also, if the spores fall in or very near the leaf axils of very young seedlings, the infection may reach the growing center and become systemic. If, however, the spores fall upon the fully differentiated, mature, and more resistant tissue of well-grown mature leaves of older plants, they will give rise to local mycelia which have only a limited restricted growth as locally infected spots. These reactions of the fungus, inferred from field observations, were not verified by inoculation experiments; but it should be noted that in the case of the conidial *Sclerosporas* of the Philippines the development under differing conditions of infection has been found, in work not as yet published, to be that described above.

The other interesting aspect of the local type of infection is its possible importance in the distribution of the disease. Although relatively inconspicuous and supporting relatively scanty spore production in comparison with the systemic type, the local infection, because of its common occurrence and its persistence throughout the

year, plays an active part in spreading the *Sclerospora*, a point that should be borne in mind in connection with the question of its economic importance and control.

## THE CAUSAL ORGANISM

### ITS IDENTITY

As was to have been expected from its restriction to gramineous hosts and the nature of its effect on these hosts, the causal organism proved to be a member of the peronosporaceous genus *Sclerospora*, a genus noted for its destructive parasitism of the Gramineae. As the fungus occurred in luxuriance, it was readily possible to follow the development of both the conidial (zoosporangial) and the oogonial stages, to collect an abundance of material of both phases, and to make the numerous measurements of sizes of spores and other structures necessary to serve as an adequate basis for identification. On this basis the fungus was determined to be *Sclerospora graminicola* (Sacc.) Schroet., a species of wide distribution, but one not reported hitherto from such Southern States as Florida or on this species of grass.

### STRUCTURE, DEVELOPMENT, AND RELATION TO ENVIRONMENTAL CONDITIONS

The mycelium of the fungus in cases of systemic infection is very generally distributed through the tissue of the host, occurring especially abundantly in the pallid areas of the symptomatically marked leaves (see pl. 1, A, D, E) and to a lesser degree in the leaf sheaths, in the inflorescences, and in the stem both above and below the ground, but not in the roots. In cases of local infection the mycelium is restricted to the tissue of the discolored spots. (See pl. 1, F.) In either case the mycelium is intercellular in position, comprises slender hyphae of transmission, running from place to place along the bundles, and knotted, much-branched, contorted, feeding hyphae which are crowded into the interstices between cells of the mesophyll and furnished with short knob-shaped or finger-shaped haustoria.

From this mycelium are developed the two successive reproductive phases of the fungus, the treelike conidiophores that grow out externally from the stomata, and the large thick-walled oogonia or resting spores that are formed later within the disintegrating host tissue.

### THE CONIDIAL STAGE

Conidiophore production on this host and under subtropical conditions in Florida, like that already described for this fungus on fox-tail grass in Minnesota (21), takes place at night when the surfaces of the mycelium-invaded tissue are covered with dew or other moisture, a relationship that has been considered in more detail in earlier papers (19, 20, 21). Preliminary to conidiophore production the mycelium among the mesophyll cells gives rise to thick, irregular clusters of hyphal branches closely crowded in the air chambers beneath the stomata from which the conidiophores will arise. The subsequent development of the conidiophores and conidia agrees with

the process as it has been described already for this *Sclerospora* on *Chaetochloa* (*Setaria*) *viridis* in Minnesota. This agreement holds in all phases of the process from its beginning with the emergence from the stomatal slit of branches which form a compact group of bulbous knobs, elongate into club-shaped stalks, and develop by further elongation and branching into the conidiophores, up to and through the final production of conidia (zoosporangia) on the terminal sterigmata of the mature conidiophore and the subsequent collapse of these when the conidia have been shed.

#### THE CONIDIOPHORES

The descriptions and drawings of all these stages of conidiophore production, as they were found to occur on *Chaetochloa* (*Setaria*) *viridis* in Minnesota (21), would serve with equal accuracy to depict the process as followed on *C. magna* in Florida. Also the distinctive mature conidiophores (fig. 4, A, B, C, D) agree in such critical morphological features as the size and shape of the main axis, with its slightly differentiated basal foot (fig. 4, E to J); the structure, number, and position of the branches; the shape and arrangement of the sterigmata, and the structure of the spores borne on them (fig. 4, K to O). This agreement is well shown if the accompanying drawings of typical, well-developed conidiophores under optimum nocturnal conditions (fig. 4) are compared with similar conidiophores illustrated for *Sclerospora graminicola* in Minnesota (21, pl. 1, 2). As in the material of *S. graminicola* studied in Minnesota, the basal cell, which is such a consistently developed feature in the Philippine *Sclerosporas*, is the exception here. (See fig. 4, E to J.)

#### THE CONIDIA

The conidia, in all the essential distinguishing features of size, shape, structure, and germination, show the characteristic peculiarities that mark *Sclerospora graminicola* as such a distinct species.

In size the conidia, like those of the downy mildews in general, show considerable individual variation. The size should be presented quantitatively, therefore, from large numbers of measurements, if it is to be adequate for identification or comparison. Taking advantage of the facilities for night work that were generously offered by Hugh Matheson at Coconut Grove, one of the writers measured 250 conidia on the nights of June 16 and 17, 1924, under optimum conditions, during maximum production from 1.30 to 3 a. m. They were measured while fresh and living in the dew in which they had been shed. In making previous diagnostic measurements of this species in Minnesota in 1920, it had been necessary (21) to use preserved material. This, although killed very carefully in Flemming's weaker solution and mounted in dilute glycerin, shrank slightly, so the conclusion was reached that "a comparison of measurements made thus with the relatively few which the writer had opportunity to make from fresh material indicates that if all the measurements had been made under ideal conditions the modes of length and of diameter probably would have been increased by one

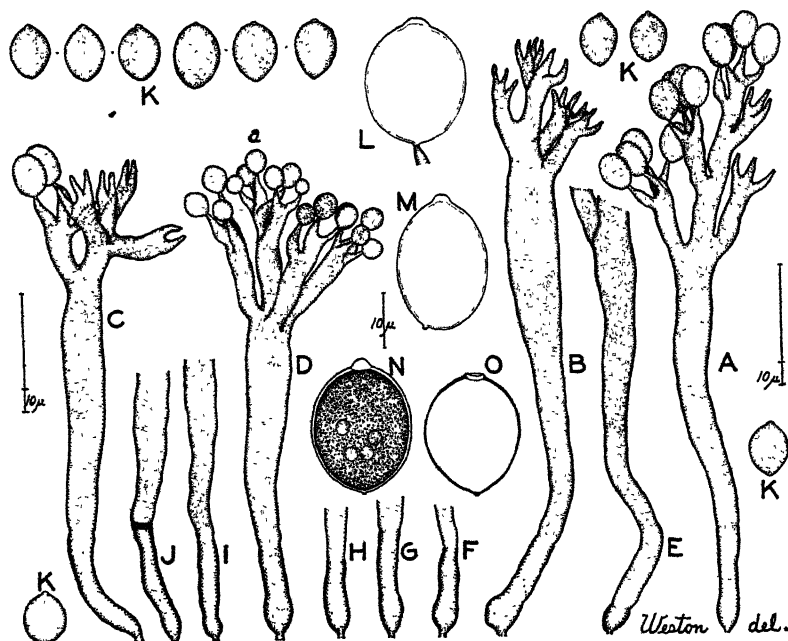


FIG. 4.—Drawings of the conidiophores and conidia (zoosporangia) of *Sclerospora graminicola* on *Chaetochloa magna*. Prepared with the aid of a camera lucida and with the exception of K (made from living spores just shed in dew) made from material scraped from leaves at the time of maximum nocturnal production of conidiophores, immediately killed with Flemming's weaker solution and stained with eosin, or killed and stained at the same time with picronigrosin; then mounted in dilute glycerin. The approximate magnification of the printed figures after reduction from the original drawings is given in each case; also a 10  $\mu$  scale is shown as an absolute measure.

A.—A conidiophore in which is especially accentuated that continuation of the main axis through the branch system which characterizes the species. The zoosporangia are only partly grown, as is obvious from their size and structure; from the two branches with truncate tipped sterigmata at the right they have been broken off.  $\times 375$

B.—A partly grown conidiophore in which the branch system is already complete and the ultimate tips have elongated into the tapering sterigmata from which the zoosporangia will bud out. As is customary in the species, the base is differentiated by thickening of the wall into a footlike portion.  $\times 375$

C.—A rather scanty conidiophore which has lost all but two of its zoosporangia and shows clearly the usual structure of the branch system with its continuing main axis.  $\times 375$

D.—A well-developed though rather stocky conidiophore which in contrast to those shown in B and C has its several branches of approximately equal size and rank coming off almost at the same point from the main axis (which can, however, be seen continuing through to a). This type of branching is occasionally encountered and represents for the species one extreme of the range of forms of which the type of A, with its pronouncedly excurrent main axis, is the other extreme.  $\times 375$

E.—Main axis of a conidiophore showing at its base an unusually short footlike differentiation.  $\times 375$

F-I.—Bases of five conidiophores showing differentiated footlike portions which in extent and thickening of the wall are typical of the species.  $\times 375$

J.—Base of a conidiophore with a complete basal cell cut off by a cross wall, a rare condition in *Sclerospora graminicola*, though typical in the conidial oriental species.  $\times 375$

K.—Ten representative conidia (zoosporangia) showing the range of shapes and sizes most frequently encountered. Note that each has the terminal papilla of dehiscence essential to its germination by the emission of zoospores.  $\times 375$

L.—A mature zoosporangium just before being shed from its sterigma, drawn in outline to show the structural modification of the wall at the tip into the dehiscence papilla and at the base into the apiculus of attachment to the sterigma.  $\times 375$

M.—Outline drawing of a mature zoosporangium recently shed from its sterigma and showing the basal apiculus of attachment located, as it occasionally is, to one side of the median line.  $\times 850$

N.—A mature, recently shed zoosporangium with its apical papilla of dehiscence well developed, its basal apiculus of attachment still discernible, and its granular content, stained with picronigrosin, still relatively undifferentiated before division into zoospores.  $\times 850$

O.—Empty wall left by such a zoosporangium as N after it has germinated by emitting zoospores through the pore left by the softening of the terminal dehiscence papilla.  $\times 850$

2  $\mu$  class (from 18 to 20  $\mu$  and from 14 to 16  $\mu$ )” (21). It is of interest that the measurements in Florida of living, freshly shed spores closely bear out this prediction, as the modes of length and of diameter fall in the 22  $\mu$  and the 16  $\mu$  classes, respectively. Florida conidia secured at the same time, but mounted in dilute glycerin, shrank slightly and gave the same smaller measurements as the preserved Minnesota material. As might be expected, since the shrinkage was approximately even, the ratios of length over diameter were not altered, the mode falling into the ratio class of from 1.25 to 1.34 in both preserved and living spores.

It is of interest that the diagnostic conidial measurements of this species on *Chaetochloa* (*Setaria*) *viridis* in St. Paul, Minn., in July, 1920, and on *C. (Setaria)* *magna* in Coconut Grove, Fla., in June, 1924, agree so closely. This agreement seems to indicate that such a quantitative expression of spore size is indeed a dependable distinguishing character of the species and not appreciably altered by development under very different climatic conditions or on different hosts. The table of spore measurements of living material here presented (Table 2) may be regarded, then, as accurately representing the conidia of *Sclerospora graminicola*.

TABLE 2.—Measurements and ratios of length to diameter of conidia (zoosporangia) of *Sclerospora graminicola* arranged in size and ratio classes, comparing those of living material from *Chaetochloa magna* in Florida in 1924 with those of preserved material from *C. viridis* in Minnesota in 1920 previously recorded by Weston (21)

Classes	Florida 1924 living material, number of conidia in 250	Minne- sota 1920 preserved material, number of conidia in 400	Classes	Florida 1924 living material, number of conidia in 250	Minne- sota 1920 preserved material, number of conidia in 400
Length:			Diameter—Continued		
11 to 12.9 $\mu$ .....	0	7	13 to 14.9 $\mu$ .....	39	162
13 to 14.9 $\mu$ .....	2	25	15 to 16.9 $\mu$ .....	105	92
15 to 16.9 $\mu$ .....	11	67	17 to 18.9 $\mu$ .....	72	30
17 to 18.9 $\mu$ .....	35	119	19 to 20.9 $\mu$ .....	22	4
19 to 20.9 $\mu$ .....	59	89	21 to 22.9 $\mu$ .....	7	0
21 to 22.9 $\mu$ .....	69	51	23 to 24.9 $\mu$ .....	1	0
23 to 24.9 $\mu$ .....	46	22	Ratio, length to diameter:		
25 to 26.9 $\mu$ .....	17	10	0.95 to 1.04.....	0	1
27 to 28.9 $\mu$ .....	6	6	1.05 to 1.14.....	11	31
29 to 30.9 $\mu$ .....	4	1	1.15 to 1.24.....	87	75
31 to 32.9 $\mu$ .....	0	1	1.25 to 1.34.....	119	115
33 to 34.9 $\mu$ .....	0	2	1.35 to 1.44.....	26	92
35 to 36.9 $\mu$ .....	1	0	1.45 to 1.54.....	4	46
Diameter:			1.55 to 1.64.....	3	25
9 to 10.9 $\mu$ .....	0	1	1.65 to 1.74.....	0	6
11 to 12.9 $\mu$ .....	4	111	1.75 to 1.84.....	0	9

It also is of interest that the few measurements of conidia from locally infected plants show a preponderance of larger sizes compared to the representative measurements just considered from systemic material. This material was comparable from the point of view of source, as it was taken from locally infected plants of approximately the same age, subjected to similar conditions as regards location, temperature, and moisture. It might, therefore, be

regarded as significant, an indication of a distinct strain of *Sclerospora graminicola*, only slightly different morphologically from the type, but decidedly different physiologically in its effect on the host. It seems doubtful to the writers, however, that this interpretation is the correct one. In the first place, as has been discussed earlier, the symptoms as studied in the fields seem much more probably to be the result of later infection of more mature parts of the plants. In the second place, the 50 measurements of conidia from local spots are too few to give an adequate basis for comparison, for if the 250 measurements of conidia from typical systemically infected plants are examined in lots of 50, some of these lots show as great a departure from the size distinctions established by the total as do the measurements of the local spot material. Moreover, as was noted above, the difference is one of slight increase equally in all dimensions and not accompanied by any difference in shape, the ratio of length divided by diameter being the same.

In shape the conidia are most frequently broadly ellipsoidal or rotund cylindrical, but they show considerable variation. A qualitative idea of the usual range is given by the illustrations of the representative shapes of 10 spores in Figure 4, K, while a quantitative conception of the frequency of the broadly ellipsoidal form (with a length from 1.25 to 1.34 times the diameter) is brought out clearly in the tabular presentation of the ratios of length over diameter. (Table 2.)

The structure of the conidia (fig. 4, L to O) agrees with that already described for the species (21) in the details of the thin cellulose wall, of the specialized apical papilla of dehiscence, and the granular content with its several nuclei.

On germination the few to several zoospores into which the content has developed escape through the terminal pore left by the gelatinizing of the apical papilla. This method, essentially zoosporangial, is the typical one by which the spores liberated during the night into dew or other moisture germinated under natural field conditions. It took place rapidly and universally at temperatures from 70° to 80° F. on leaves or other plant parts, or on slides in drops of dew, rain, or tap water. At 50° in an ice box it was the only method obtaining in the very low percentage of slow germination that did occur. At 90° or 92° no germination took place and the content of the spores became somewhat clotted and vacuolate and seemed irreparably injured. Very rarely, in exceedingly few cases of the large number observed, the spores germinated by putting out a short abortive hypha, presumably in response to conditions unfavorable for normal germination, as the few instances occurred in the laboratory in the mounts that became unduly warm (85° plus) when observed over a substage lamp and in material collected in nature in scanty moisture on leaves drying in a warm breeze at dawn.

#### THE OOGONIAL STAGE

In Florida, on the Everglade millet, as elsewhere in the world on other hosts, the life cycle of this downy mildew involves an oogonial as well as a conidial stage, both stages usually occurring in the life cycle with neither one developing to the exclusion of the other.

The occurrence of the oogonial stage usually follows the conidial, hence the oogonia customarily develop on the lower, older leaves after the formation of conidia thereon has ceased, even though the latter still may be developing on the upper, more recently expanded leaves of the same plants. Occasionally, however, the oogonia may develop in profusion together with the conidia on the same leaf; and usually for a time during the transitional period when any leaf is ceasing to develop conidia and beginning to form oogonia, some of each may be found upon and in it.

The oogonia are developed within the tissue of the host, chiefly in the leaves, but to a less degree in the leaf sheaths, or in the modified leaflike structures which form in malformed badly infected heads. (Pl. 2, D.) On these parts of the plant the oogonia are formed by the mycelium chiefly where it is most abundantly developed between the disjoined cells of the spongy mesophyll and of the bundle sheaths around the fibrovascular bundles. (Pl. 2, E, F.) As the formation of the oogonia proceeds, the host cells in these regions are forced apart, crushed, and destroyed progressively until ultimately through this disorganization and withering of their tissue the leaves become shredded by longitudinal splitting between the bundles, leaving these as delicate, threadlike fibers, at first more or less parallel in a brush-like arrangement, but later tangled and snarled into knotted masses. (Pl. 2, C.) From the disintegrating interfascicular tissue the oogonia, which by now are mature, shake out and are scattered very effectively by even the slightest quivering of the plant in the wind. So characteristic is the shredding of the leaves in the latter stages of the disease that it is one of the most distinctive symptoms of this downy-mildew infection of Everglade millet in Florida, as it is on other hosts in other parts of the world.

The formation of the oogonia by the mycelium within the tissue agrees in its general features with the early description of Schroeter (13) in the case of *Setaria viridis*, and of Butler (2) in *Pennisetum typhoideum*. By cutting sections or macerating the tissue, the swelling of the terminal or intercalary oogonia and the attachment of antheridia from near-by branches of the mycelium were easily followed, the process in gross details and in its minute cytological features agreeing closely with the careful account given by Stevens (17) for this same species in *S. viridis*.

The oogonia when fully mature are quite distinctive. In color they range from a somewhat pallid amber through deeper reddish tones approximating the color of dark rosin, or, according to Ridgway's (11) color scheme, from pale ochraceous orange, through zinc orange and xanthine orange, to orange rufous and amber brown. Perhaps the most common coloration is that of rather dark reddish rosin, which agrees rather closely with the shade called orange rufous by Ridgway. The single oospore within the oogonium, on the contrary, has a somewhat pallid, almost hyaline wall, sometimes slightly tinged with a pallid golden or amber tone. The content has chiefly the pallid, somewhat gray shade of protoplasm, but may contain here and there oil globules that are generally slightly golden.

In shape the oogonia vary greatly, being in general somewhat rounded polygonal, their outlines conforming largely to the shape of

the intercellular spaces in which they have formed. (Pl. 2, F.) The oospore itself, however, is approximately spherical with but little variation in shape in large numbers of spores examined. (Cf. pl. 2, G.)

In structure the oogonium is characterized by the heavy uneven wall that led Schroeter to give the genus its distinctive name. On the outside this shows an irregular roughness, due partly to firmly adhering portions of the modified walls of disintegrated mesophyll cells among which the oogonia formed and partly to ridges developed on the wall of the growing oogonium as it was forced into the interstices between the surrounding cells. The true wall of the oogonium itself is thick but uneven, from 2 to even 10  $\mu$  in thickness, somewhat folded or irregularly dented and protruded, and of a hard, rather impermeable material greatly modified in composition from the original fungous cellulose. The inner surface of this wall is rather irregular in contour so that in some points it touches the spherical oospore inclosed and in others lies at a little space from it. (Pl. 2, G.) The wall of the oospore, however, is rather even, usually about 2  $\mu$  in thickness, smooth, refractive, and clear (pl. 2, G), and to various reagents reveals a different chemical composition from the wall of the oogonium surrounding it. Here and there the wall of the oogonium apparently has fused or become cemented to the wall of the inclosed oospore, whereas at other points there quite obviously is a gap between the outer surface of the oospore wall and the inner surface of the enveloping oogonium. After softening the oogonia with KOH and crushing them slightly, the oospores can be forced out entire in some instances, but ordinarily they remain securely inclosed by the oogonial wall even after lying in moist soil or soaking in water for weeks or months. Obviously, in the case of such bodies as these, in which the inclosing oogonium so closely envelops the single oospore within, the whole body must be considered as an entity and a resistant spore in its entirety quite as Schroeter pointed out in establishing the genus.

The size of these resting spores is a distinguishing feature which marks off this species clearly from most of the larger oogonial forms that have been described. In the size of the oogonium itself there is considerable variation, and because of the irregularity in shape of the whole structure, it is difficult and not particularly valuable in diagnosis to establish the size limit through quantitative measurements.

The inclosed oospore, however, shows much less variation in size, and since with proper illumination it can be seen even when the enveloping oogonial wall is thick and irregular, the diameters of the oospores can be measured with considerable accuracy. Such measurements of a large population of representative oospores furnish a good basis for comparing this species with others. The tabulation of frequency distribution of the various sizes encountered, as shown in Table 3, gives, therefore, a fair quantitative as well as qualitative conception of spore size as a diagnostic characteristic of the species.



TABLE 3.—Measurements of diameters of the oospores of *Sclerospora graminicola* on *Chaetochloa magna* plants in Florida, tabulated both for separate collections made at different times in different localities and for the total

Classes	Cocoplum Beach Road, 1924; number of oospores in 250	Key Biscayne, 1924; number of oospores in 250	Bird Road garden, 1924; number of oospores in 350	City of Sarasota, 1927; number of oospores in 150	Total number of oospores in 1,000
Diameter:					
19 to 20.9 $\mu$ .....		1			1
21 to 22.9 $\mu$ .....		1	1		2
23 to 24.9 $\mu$ .....		3	5	1	9
25 to 26.9 $\mu$ .....	3	6	7	6	22
27 to 28.9 $\mu$ .....	11	29	10	21	71
29 to 30.9 $\mu$ .....	36	66	37	31	170
31 to 32.9 $\mu$ .....	61	61	59	38	219
33 to 34.9 $\mu$ .....	46	45	58	29	178
35 to 36.9 $\mu$ .....	42	19	68	15	144
37 to 38.9 $\mu$ .....	27	15	55	6	103
39 to 40.9 $\mu$ .....	21	4	36	3	64
41 to 42.9 $\mu$ .....	1		9		10
43 to 44.9 $\mu$ .....	2		5		7

On comparing the measurements with those given by Schroeter (13), by Saccardo (12), by Fischer (5), by Berlese (1), by Traverso (18), by Butler (2), by Shirai (15), and by Ideta (6), they are found in general to agree, though it should be noted that most of the oospores measured by the writers fall within the size limits of 30 to 36  $\mu$ , which is not the average but rather the larger size limits of the relatively few measurements of the inclusive qualitative sort made by earlier investigators mentioned above.

In view of the fact that *Sclerospora graminicola* has such a diverse range of hosts and occurs so very widely distributed throughout the world, it would be of considerable interest to make an intensive comparative study of large numbers of oospores from many collections on different hosts and from different localities, to determine whether the species comprises strains with slight morphological differences in size and structure, as in the case of some other genera of downy mildews. Such a comparative investigation has been undertaken by the senior writer but has not progressed far enough to justify drawing conclusions as yet. It would be equally desirable to make a similar comparison of the zoosporangia, but as they should be measured at once when shed under optimum conditions, or at least should be carefully preserved under such conditions for future study, such an investigation is rather impracticable at present. The comparison in Table 2, however, between zoosporangia of this downy mildew on green foxtail grass in Minnesota in 1920 and on Everglade millet in Florida in 1924 shows no indication of morphologically distinct strains on these hosts in these two widely separate localities in the United States.

Although the structure, development, and general characteristics of the oogonia were determined without undue difficulty as they occurred on this new host in Florida, and although they have been described, at least in part, by other investigators of this species on

other hosts, the part these spores play in the life history of the fungus has not been worked out in all its details, and the actual method of germination has not been reported or investigated. As infection of seedlings in earth containing oospores was secured by the junior writer in the fall of 1924 in inoculation tests described later in this paper, it is justifiable to assume that germination of these bodies does take place in nature, and the evidence in the field is strong that it is occurring regularly in Florida under ordinary climatic conditions from year to year. The work of Melhus and Van Haltern (9) on the inoculation of various hosts by means of the resting spores, and more recent investigations by these authors and Bliss (10) on the hibernation and resistance of these bodies have added greatly to our knowledge of the oosporic phase of the fungus and have given information as to its activities which probably applies under Florida conditions as well as those of Ames, Iowa.

#### DISSEMINATION AND PERSISTENCE OF THE PARASITE

The dissemination of such fungi as this *Sclerospora* in general is of the two types recognized by Butler (2, 3), a local noncontinuous spread by successive short flights and a long-distance spread in continuous uninterrupted journeys over great areas of land or water. In *Sclerospora graminicola* distribution of both types is known to occur, although in Florida it is chiefly the local spread from field to field and from locality to locality that seems to be taking place.

In the dissemination of the fungus the zoosporangia (conidia) and the zoospores emerging from them, the resting spores (consisting of the oospore within the enveloping oogonium), and even the mycelium itself may take part. In a study of the various aspects of the distribution and severity of the disease in Florida and the resultant measures for its control, the question of the structures taking part in dissemination and in persistence is of especial interest.

Local dissemination from plant to plant by the zoosporangia (conidia) apparently takes place very effectively. In Florida the production of abundant zoosporangia occurs practically throughout the year, for although it is at its maximum during the moist, cool nights of late winter and early spring, there is no time of year, at least in the southern part of the east coast, when in the infected clumps of grass one fails to find plants on which some zoosporangia are being produced in a practically continuous supply.

Dissemination of the fungus apparently is accomplished even more generally and successfully by means of the resting spores. These resting spores are produced, as has been said, in the parts of the plant in which the mycelium of the fungus is maturing, and as the grass clumps in any region are of different ages and new shoots are being sent out successively, there is a continuous production of the resting spores throughout the year, as there is of the zoosporangia, whose formation usually precedes them on the leaf surface.

Dissemination by means of mycelium within the host seemingly does not play any part in the case of this downy mildew, for the Everglade millet is propagated regularly not by stem cuttings which, as in the case of sugar cane, would contain living hyphae, but rather by seed. Apparently the mycelium does not become enscathed in

the seed, for when it grows so extensively in the head as to penetrate the developing ovaries these are badly deformed, and the seeds if formed at all are so imperfectly developed that they do not germinate.

The agents which accomplish dissemination of the fungus in the form of the zoosporangia and resting spores just considered are primarily wind and secondarily water. Although no special experiments were made to follow the distribution of the zoosporangia, this apparently involves the same general procedure as that already studied (20) in the case of the Philippine *Sclerosporas*, the conidia on maturing being wafted by the wind from the surface of the leaves on which they are produced and carried by moist night breezes to near-by plants. In the case of the resting spores wind is very active in distribution also. As the leaves in which the resting spores are maturing become shredded from the disintegration of the tissue between the fibrovascular bundles, these, being somewhat hygroscopic, twist and curl as they absorb moisture or as they dry out, effectively freeing the spores between them even on quiet days, and shaking with even the slightest jar of almost imperceptible breezes, so that a more or less abundant shower of resting spores is being scattered continually. Slides exposed under such plants caught an abundance of spores, and, as they are relatively small and light, it is justifiable to assume that they are carried away easily by the wind.

No matter how successfully these two types of reproductive bodies might be distributed, however, they would not achieve the spreading of the fungus unless they could accomplish the infection of healthy plants to which they might be blown. As has been noted, the zoosporangia are relatively short-lived and will not withstand drying or freezing or even brief exposure to strong sunlight. As they are produced at night, however, during abundant dew formation, their production occurs under conditions ideally suited for their being spread from plant to plant and reaching new hosts, still living and able to germinate and cause infection. These zoosporangia when caught on slides at night while they were being shed were found to germinate rapidly in pure water or in the dew that covered the leaves of near-by susceptible plants.

The resting spores, however, in spite of repeated observations, have not been seen to germinate, and their method of so doing and the exact process by which they accomplish infection are unknown. That they do germinate and do accomplish infection seems not to be doubted, however, both from observations in the field in Florida and from the following experiments by the junior writer. Series of tests were made in which healthy seeds were collected and planted in flats of sterilized soil with which had been mixed dried remains, 9 months old, of plants bearing an abundance of resting spores. The seeds germinated vigorously in about 8 days, and the seedlings appeared and reached a height of several inches in 2 weeks. When the seedlings were 4 weeks old they were examined carefully and 15 per cent were found to have been killed by the downy mildew, while 90 per cent of the survivors showed abundant infection. It is possible, of course, that resting mycelium in the dried plant parts mixed with the soil was responsible for this infection, but microscopic examination of such tissue gives no evidence that the mycelium is structurally

adapted to such resistance, and it seems much more probable that infection resulted from the germination of the resting spores thus introduced into the soil. It must be admitted, however, that careful searching and the examination of hundreds of resting spores taken from such soil or kept in hanging-drop cultures never revealed one that had germinated or showed any changes indicative of germination.

In addition to the means by which the fungus spreads from plant to plant and from place to place, the methods by which it persists from season to season and from year to year also are of great importance in their relation to the possible destructiveness of the disease in Florida. In connection with the persistence of the fungus from season to season, the same zoosporangia, resting spores, and mycelium concerned in its distribution must be considered.

The mycelium once ensconced in the tissue of the host remains there. In many cases, especially if the plant is fairly mature when attacked, the host is not killed but goes on developing and putting out new shoots, which in turn are invaded by the mycelium, so that this extension and this persistence of the parasite continue uninterrupted through the season. As has been noted, moreover, the Everglade millet, at least in the more southern parts of Florida, is not limited to a short growing season but is perennial, its clumps continuing to grow and to develop new suckers more or less vigorously from month to month and year to year. In such of these perennial clumps as are infected the persistent mycelium of the mildew also lives on indefinitely, running out into the newly developed shoots, where it sends forth successive crops of conidiophores on favorable nights and persisting and maturing resting spores throughout the tissue that is growing older. In some cases the infected plants seemed to be stimulated to much more active perennial growth than the uninfected, vigorously continuing to send out new shoots during the cold or dry periods when the activity of adjacent healthy plants was very slight. By the mycelium, therefore, the continuance of the mildew is very generally accomplished.

The zoosporangia are also instrumental in securing the persistence of the parasite from year to year under Florida conditions, for while they themselves are not resistant and do not survive long as such, they are produced in crop after crop by successive growth of the mycelium and continually accomplish infection and reinfection through the vicinity, thus serving as a means of perpetuating the disease.

Apparently, however, it is the resting spores especially that perpetuate the mildew from year to year. In contrast to the mycelium, which can not continue except as nourished by the host, and to the sporangia, which must reach and infect new hosts almost immediately, the resting spores are able to survive in plant remains and in the soil for considerable periods of time. The tissue-bearing resting spores which were mixed with earth for the infection experiments mentioned above had been dried in some cases for as long as 17 months and yet produced infection in the young seedlings in the same high percentage of cases as those more recently gathered. There is every evidence in the field that the part played by the resting spores is very important in the persistence of the disease in Florida. Obviously,

when one appreciates these various means by which the fungus is so efficiently distributed and perpetuated, it is easy to understand the persistently high percentage of infection of the Everglade millet throughout Florida. This matter also involves the question of the effectiveness of the methods by which infection is accomplished and of the rapidity with which infection is followed by spore formation and reinfection in building up an epidemic.

The precise method by which infection of the host plants is secured has not been studied in detail in Florida. It seems justifiable to assume, however, from what has been found to occur in the case of the Philippine *Sclerosporas*, that in this species also infection by means of the zoosporangia (conidia) and the zoospores emerging from them occurs, first, through the stomata in the young and tender tissue both of the curled-up terminal leaf and of the growing point within it surrounded by dew and exuded moisture, and, second, through the stomata of young leaf axils holding drops of dew. The manner in which the resting spores bring about infection has not yet been worked out, nor is it known whether the resting spores emit zoospores or send out a hypha that penetrates the host directly, or a limited mycelium which bears zoosporangia. The experiments by the junior writer reported here and the successful inoculation of maize and other hosts with these bodies by Melhus and Van Haltern (9) leave no doubt that infection does occur by means of resting spores. Moreover, it is clear from some of these cases that infection of germinating seedlings of the host plants had taken place even before they emerged from the ground.

The period of incubation elapsing between the time of infection and the production of zoosporangia or oogonia by the mycelium that has invaded the tissue is not known. In the experiments reported above, the seedlings showed infection and even produced sporangia in from 2 to 4 weeks after emerging from the soil containing resting spores. If one may judge from the case of the Philippine maize mildew, the incubation period following conidial infection would be even shorter, for infection of young seedlings by that mildew gave rise to mycelium which invaded the tissue very rapidly and in as short a time as 3 to 10 days produced abundant crops of spores on the surfaces of the young leaves. Even the longer period of 2 to 4 weeks, however, is sufficiently short to explain adequately the rapid increase in extent and amount of infection of the Everglade millet under Florida conditions.

#### PATHOGENICITY

The question whether the downy-mildew disease, so widespread, so virulent, and so successfully persistent on its wild host, the Everglade millet in Florida, may spread to valuable grasses cultivated as crops throughout the State is one of importance.

The crops which this species of downy mildew has been found to attack during the years it has been known in various parts of the world are chiefly Italian millet (*Chaetochloa* (*Setaria*) *italica* (L.) Scribn.) and the closely related Hungarian millet (*C. italica germanica* (Mill.) Scribn.), the bullrush or pearl millet (*Pennisetum typhoides* L.), and the proso or broom millet (*Panicum miliaceum*

L.). The disease has been reported also as destructive to sorghum (*Andropogon sorghum* L.) in India, although it should be noted that there are strong reasons, which have been pointed out already (20), for believing that the fungus causing it is really a different species. Moreover, according to the not wholly convincing report of Massee (8), the disease has occurred, though not injuriously, on sugar cane (*Saccharum officinarum* L.) in England. Finally, the disease has been found to attack maize or Indian corn. This was first reported by R. B. Streets, who, in the summer of 1921, found the disease on sweet corn in Wisconsin and described his investigation of the situation in his report<sup>5</sup> to the Office of Cereal Crops and Diseases of the Bureau of Plant Industry. Recently Melhus and Van Haltern (9) have been successful in artificially infecting with *Sclerospora graminicola* not only the cultivated millets, *C. (Setaria) italica* and *P. miliaceum*, but also teosinte (*Euchlaena mexicana* Schrad.) and 26 varieties of maize.

In addition to these cultivated plants, the wild grasses, green foxtail (*Chaetochloa (Setaria) viridis*), and the smooth foxtail (*C. glauca*) have very generally been found to be attacked by this downy mildew wherever they occur in Europe and America.

Of all these host plants those that previous records show to be most susceptible, namely, the species of *Chaetochloa*, both the cultivated (the Italian millet and Hungarian millet) and the wild (the green foxtail and the smooth foxtail grass) are very rare in Florida and have not been available for examination.

In the case of pearl millet and sorghum, which are grown rather commonly in small patches, a careful examination of representative fields, chiefly around Miami, revealed no instances of attack by the downy mildew, even though plants of Everglade millet in all stages of infection by the fungus were growing abundantly in and around the cultivated areas.

Also, in the case of sugar cane, all the evidence gathered from field inspection indicates that the fungus certainly is not virulent to cane under field conditions in Florida. For example, along the edges of cane fields of plantations west of Miami, especially during the summer of 1924, there were great numbers of Everglade millet plants heavily infected with the downy mildew and obviously exposing to infection the young cane plants only a few feet away, yet careful search revealed no cases of downy mildew on the cane.

Furthermore, in the case of maize, a careful examination of fields in different districts in the State in which plants of the Everglade millet of all ages were growing in profusion as weeds, and were infected practically without exception with the actively sporulating downy mildew, did not reveal a single maize plant that was infected. In one cornfield near Miami, for example, the Everglade millet in 1925 was found growing abundantly as a weed among the corn, the grass even growing in the hills with the maize seedlings. When first examined the intermingled seedlings of the grass and the corn were young, some inches tall, in many instances with their leaves

<sup>5</sup> As this report and Streets's subsequent reports of his later survey work on this disease for the Office of Cereal Crops and Diseases were not published, they have escaped the attention they deserve, and the senior writer therefore has prepared a brief account of them, which is expected to appear shortly.

touching each other, and over 90 per cent of the grass seedlings were diseased with the downy mildew. Three weeks later 100 per cent of the grass seedlings were thus diseased, and in some cases the conidia of the fungus shed from the grass had fallen on the leaves of the corn in such numbers as to be obvious as a deposit of grayish color. The field was kept under observation until the corn was mature, yet during these three months not one corn plant was found infected with the mildew.

Not only did the disease fail to infect maize under field conditions, but, in the few attempts made, it failed also to transfer to maize when more directly exposed under artificial conditions. For example, in June, 1924, at Miami, seeds of yellow flint corn locally grown, when planted in tins in which heavily downy-mildewed plants of Everglade millet were grown for a study of the nocturnal production of conidia, developed into healthy seedlings, although from their first emergence they had been exposed repeatedly to showers of spores from the millet above them—a procedure that in the case of the Philippine *Sclerosporas* of maize would have resulted with certainty in a heavy percentage of infection.

The foregoing observations, of course, are too few and limited to be conclusive. They do indicate strongly, however, that cases of passage of the disease from Everglade millet to the crops mentioned are apparently very rare and certainly not numerous under Florida conditions.

#### ECONOMIC IMPORTANCE

At present the downy mildew as it exists in Florida is not of any great economic importance. The Everglade millet which is attacked is not sufficiently troublesome as a weed or sufficiently valuable as an uncultivated forage plant to make its destruction by the mildew either agriculturally a gain in the one case or a loss in the other.

However, the question to be considered is whether the disease in the future may not prove to be much more important. The present situation, that of a virulent disease with great potentialities for destruction, established on an abundant wild grass through all parts of the State, presents the alarming possibility that these infected areas of grass, although valueless in themselves, may serve as endemic foci of infection from which the disease may spread to related cultivated crops of great value, causing seriously destructive epidemics that could be controlled only at great cost. With this, as with other downy mildews, the destructiveness to various susceptible crops would depend not only on the power of the parasite to injure and destroy individual plants but also on the ability of the fungus to spread rapidly to large numbers of individuals. Obviously, in any given region such a disease might be rapidly fatal to individual plants yet not be of economic importance if local conditions did not permit the rapid spread to vast numbers of individuals; or a disease both severe and rapidly spreading would be relatively unimportant in a locality in which the crops thus attacked were of little value. In the case of this downy mildew in Florida, there is no doubt of its serious injury to individual plants, especially young ones, and no doubt of its effectiveness in rapid multiplication and spread. The question, therefore, is one of the present and possible future value

of the crops attacked. The Everglade millet on which it occurs is of no value in itself, as has been said, but its occurrence and that of the coextensive downy mildew upon it are so widespread throughout the State that it must always be considered as a potential reservoir of infection for susceptible cultivated crops.

As has been mentioned, the crops which have been reported as most susceptible to the disease elsewhere in the world are the Italian millet, the Hungarian millet, and the pearl millet, while there are records also of occasional occurrence on proso millet, sorghum, teosinte, sugar cane, and corn. Of these crops Italian millet and its variety, Hungarian millet, pearl millet, proso millet, sorghum, and teosinte are but little grown in Florida, and it does not seem probable that in the future they will be developed to such an extent that they will become of great value agriculturally.

Sugar cane, however, grown on the necessarily large acreages of a few recently developed sugar plantations, represents considerable capital, while corn, chiefly of the hard, small, yellow or white flint type, is very commonly grown throughout the State, the acreage and value in the aggregate making the crop of importance.

Practically everywhere that these susceptible crops are grown the Everglade millet grows also, either in patches of considerable extent in waste or abandoned land, along low bottoms or roadsides, or scattered in clumps and small groups as weeds in the cultivated fields themselves. And wherever the Everglade millet occurs the downy mildew is found on it, frequently attacking from 90 to 100 per cent of the plants and from season to season very efficiently persisting, reinfecting, and spreading.

Although many fields of these susceptible crops, with the exception of a few of the less common, have been examined carefully in various parts of the State, no cases of the spread of the disease to cultivated plants have been found.

It is conceivable that this downy mildew, which, like others, is very responsive to climatic conditions, might in the future during some extraordinarily favorable season transfer to related cultivated crops and become seriously destructive to them. This, however, seems very improbable. The disease shows every indication of having been established in Florida for a long time, yet there is no evidence of any such epidemic in the past. While the disease since its discovery in 1922 has been under special observation throughout the State under the wide range of seasonal conditions of the past five years, there have been found no cases of its even transferring to cultivated crops, much less threatening to become epidemic on them. If in the agriculture of the State the future brings some radical and unexpected change, such as the extensive development of the very susceptible German millet as a valuable large-scale crop, the disease may prove troublesome. Otherwise, considering all the evidence available, it seems to the writers logical to expect that the disease will continue to be as unimportant as it is at present.

#### CONTROL

From the fact that the Everglade millet on which the mildew occurs is of no especial value, from the fact that under field condi-



tions in Florida the fungus is rarely, if at all, pathogenic to commonly cultivated crops, and from the fact that the present status of the disease and the trend of agriculture in the State indicate that the disease is likely to continue to be of negligible economic importance, it follows that the problem of control is not an immediately pressing one. But because the fungus is so widely distributed throughout the State in centers of infection where it maintains itself on the Everglade millet successfully and in profusion year after year, it is safe to predict that its control, if ever it should become necessary, will be a matter of considerable difficulty.

#### SUMMARY

The downy mildew disease of the Everglade millet (*Chaetochloa magna*), caused by *Sclerospora graminicola*, was first encountered at Vero Beach, Fla., in November, 1922. Since then it has been found to be present on this grass, both growing wild in the Everglades and growing as a weed in cultivated land, in the following 14 counties: Dade, Broward, Palm Beach, Martin, St. Lucie, Indian River, Brevard, Volusia, Putnam, Duval, Alchua, Marion, Manatee, and Sarasota. This disease has never before been found in the southernmost States, nor has it ever been reported previously on this host.

Under Florida's subtropical conditions and on this grass the fungus runs through the same conidial and then oosporic stage characterizing it elsewhere on other hosts. The conidial phase shows the same development at night when the surface of the leaves is covered with dew or other moisture, follows the same schedule of emergence and development of the conidiophores, and agrees in the structure of the conidiophore and in the structural peculiarities, method of germination, and size characteristics of the conidia (zoosporangia). In like manner the oosporic phase agrees in the course of its development between the fibrovascular bundles until the leaves shred out to fibrous tangles, and in the essential structural features of the inclosing oogonia and the structure and size characteristics of the oospores within. Quantitative measurements of 250 conidia (zoosporangia) and of 1,000 oospores as given in tabular form.

The attack of the fungus usually results in the conspicuous systemic infection commonly reported, and from this the grass does not recover, new shoots being invaded successively as they sprout. Also there was found to occur a local type of infection, hitherto unreported, manifesting itself in an inconspicuous scattered spotting on the leaves. As this is not particularly noticeable, it seems probable that it may occur elsewhere on other hosts and have escaped attention previously. This local infection is of considerable importance in accomplishing dissemination of the disease. These two types of infection are illustrated and the pattern and extent of their sporulating areas are tabulated and compared.

The part played by the conidial and the oosporic phases of the disease in its local and long-distance spread, in its persistence from year to year, and in its injury to the host is considered, and the importance of the oospores is emphasized. Experiments are reported in which infection of Everglade millet seedlings was secured by

oospores even when these had rested previously more than nine months.

Although this mildew has been reported on other gramineous hosts, both wild and cultivated, throughout the world, careful searching in Florida for over four years has not revealed it on any host but the Everglade millet. Since, wherever this grass is known to occur in Florida, the fungus has been found on it, it is suggested that elsewhere throughout its range in the Gulf States and the West Indies the host will be found to harbor this mildew. Because this mildew hitherto has been observed in the United States only on wild or cultivated hosts introduced from Europe, it has been assumed that the fungus, originally described from Europe, is of the same origin, but the fact that it occurs so widely distributed in Florida on this wild-growing native grass is possible evidence against this assumption.

The disease, in nearly five years' observation, has not been found to be of any economic importance in Florida, nor has it given indication of probability that it will become so in the future, unless, perhaps, some change to valuable plantings of very susceptible crops takes place in the State. But, as the mildew has serious potentialities for destruction, it should be kept under observation.

#### LITERATURE CITED

- (1) BERLESE, A. N.  
1897-1904. SAGGIO DI UNA MONOGRAFIA DELLE PERONOSPORACEE. Riv. Patol. Veg. 6: 78-101, 237-268; 7: 19-37; 9: 1-126; 10: 185-298, illus.
- (2) BUTLER, E. J.  
1907. SOME DISEASES OF CEREALS CAUSED BY SCLEROSPORA GRAMINICOLA. India Dept. Agr. Mem. Bot. Ser., v. 2, no. 1, 24 p., illus.
- (3) ———  
1917. THE DISSEMINATION OF PARASITIC FUNGI AND INTERNATIONAL LEGISLATION. India Dept. Agr. Mem. Bot. Ser. 9: 1-73.
- (4) FARLOW, W. G.  
1884. ADDITIONS TO THE PERONOSPOREAE OF THE UNITED STATES. Bot. Gaz. 9: 37-40.
- (5) FISCHER, A.  
1892. PHYCOMYCETES. 505 p., illus. Leipzig. (Rabenhorst, L. Kryptogamen-Flora, Bd. I, Abt. 4.)
- (6) IDETA, A.  
1914. HANDBUCH DER PFLANZENKRANKHEITEN JAPANS. Ed. 4, 930 p., illus. Tokyo.
- (7) KULKARNI, G. S.  
1913. OBSERVATIONS ON THE DOWNY MILDEW (SCLEROSPORA GRAMINICOLA (SACC.) SCHROET.) OF BAJRI AND JOWAR. India Dept. Agr. Mem. Bot. Ser. 5: 268-273, illus.
- (8) MASSEE, G. E., and MASSEE, I.  
1913. MILDEWS, RUSTS AND SMUTS. A SYNOPSIS OF THE FAMILIES, PERONOSPORACEAE, ERYSIIPHACEAE, UREDINACEAE AND USTILAGINACEAE. 229 p., illus. London.
- (9) MELHUS, I. E., and VAN HALTERN, F.  
1925. SCLEROSPORA ON CORN IN AMERICA. Phytopathology 15: 720-721.
- (10) ——— VAN HALTERN, F., and BLISS, D. E.  
1927. A STUDY OF THE DOWNY MILDEW, SCLEROSPORA GRAMINICOLA (SACC.) SCHROET. (Abstract) Phytopathology 17: 57.

- (11) RIDGWAY, R.  
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.
  - (12) SACCARDO, P. A.  
1882. FUNGI VENETI NOVI V. CRITICI V. MYCOLOGIAE VENETAE ADDENDI. SERIES XIII. *Michelia* 2: 528-648.
  - (13) SCHROETER, J.  
1879. PROTOMYCES GRAMINICOLA SACCARDO. *Hedwigia* 18: 82-87.
  - (14) SCRIBNER, F. LAMSON, and MERRILL, E. D.  
1900. STUDIES ON AMERICAN GRASSES. THE NORTH AMERICAN SPECIES OF CHAETOCHLOA. U. S. Dept. Agr., Div. Agrostol. Bul. 21, 44 p., illus.
  - (15) SHIRAI, M.  
1897. NOTES ON THE FUNGOUS DISEASES OF SETARIA ITALICA. *Bot. Mag. [Tokyo]* 11: 25-29, illus.
  - (16) SPEGAZZINI, C.  
1909. MYCETES ARGENTINENSES. SERIES IV. *An. Mus. Nac. Buenos Aires* (3) 12: 257-458, illus.
  - (17) STEVENS, F. L.  
1902. STUDIES IN THE FERTILIZATION OF PHYCOMYCETES. *Bot. Gaz.* 34: 420-425, illus.
  - (18) TRAVERSO, G. B.  
1902. NOTE CRITICHE SOPRA LE "SCLEROSPORE" PARASSITE DI GRAMINACEE. *Malpighia* 16: 280-290, illus.
  - (19) WESTON, W. H., JR.  
1920. PHILIPPINE DOWNY MILDEW OF MAIZE. *Jour. Agr. Research* 19: 97-122, illus.
  - (20) ———  
1923. PRODUCTION AND DISPERSAL OF CONIDIA IN THE PHILIPPINE SCLEROSPORAS OF MAIZE. *Jour. Agr. Research* 23: 239-278, illus.
  - (21) ———  
1924. NOCTURNAL PRODUCTION OF CONIDIA BY SCLEROSPORA GRAMINICOLA. *Jour. Agr. Research* 27: 771-784, illus.
  - (22) ——— and WEBER, G. F.  
1926. DOWNY MILDEW (SCLEROSPORA GRAMINICOLA (SACC.) SCHROET.) ON THE EVERGLADE MILLET (CHAETOCHLOA MAGNA (GRISEB.) SCRIBN.) (Abstract) *Phytopathology* 16: 71.
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# INHERITANCE OF RESISTANCE TO RUSTY BLOTCH IN BARLEY<sup>1</sup>

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## INTRODUCTION

Rusty blotch of barley, although having much of the appearance of a physiological disease, has been definitely determined to be caused by a fungus identified as *Helminthosporium californicum* Mackie and Paxton (10).<sup>3</sup> Rusty blotch has spread from isolated areas to all parts of California in which barley is a major cereal crop. Damage from this disease is expressed in excessive leaf pruning, premature ripening, blasted florets, lodged plants, and shriveled grain. (Figs. 1 and 2.) The present paper deals with the inheritance of resistance to rusty blotch in a cross between a susceptible and an immune variety of barley. Breeding varieties resistant to disease is the only complete remedy now known for the control of rusty blotch of barley.

## INHERITANCE OF RESISTANCE TO RUSTY BLOTCH IN A CROSS BETWEEN AN IMMUNE AND A SUSCEPTIBLE VARIETY OF BARLEY

After several years of experiment and observation, a variety of barley was found which for a number of years had shown no attack from rusty blotch. This barley, Chevalier, is a two-rowed variety included in the species *Hordeum distichon* (11). Chevalier was crossed with a very susceptible two-rowed variety, Abyssinian. Abyssinian is grouped by Harlan (6) under the species *H. deficiens* (11) and by Körnicke (8) under *H. distichon*, but by Beaven (2) under *H. decipiens* Steud.

Rusty blotch has occurred naturally at both Davis and Berkeley, where barley has been grown under close observation for years. In these localities Chevalier is apparently immune and Abyssinian is always susceptible.

The F<sub>1</sub> generation of the cross Abyssinian (susceptible) × Chevalier (immune) and the reciprocal were found to be completely free from rusty-blotch attack.

The F<sub>2</sub> generation was grown mainly at Berkeley because there late or spot blotch (*Helminthosporium sativum*) attacked with much less severity. Both rusty-blotch and spot-blotch lesions may, and frequently do, occur on the same leaves, rendering inspection for species disease attacks tedious and difficult. Blotches caused by mildew (*Erysiphe graminis* DC.) still further complicate a difficult situation. Mildew always attacks from the dorsal side. An examination of a leaf attacked

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 975.



FIG. 1.—Rusty blotch on the dorsal and ventral sides of leaves on the Abyssinian variety of barley. Very late attack. Berkeley, Calif.

by mildew will show but slight browning on the ventral side beneath the mildew lesion. Rusty-blotch and late-blotch lesions are more equally intense in both dorsal and ventral sides of the leaf (9).



FIG. 2.—Rusty blotch on the upper leaves of barley, causing weakening of the culm and shriveling of the grain. El Toro, Orange County, Calif.

Because of the variability in number and size of rusty-blotch lesions no separation of susceptible hybrid plants into groups was made. The separation of  $F_2$  plants into immune and susceptible groups is given in Table 1.

TABLE 1.—Segregation of the 860  $F_2$  plants obtained in a cross between Chevalier, a variety immune to rusty blotch, and Abyssinian, a susceptible variety

Item	Number of $F_2$ plants immune to rusty blotch	Number of $F_2$ plants attacked by rusty blotch
Observed.....	218.00	642
Expected.....	215.00	645
Deviation.....	+3.00	-3
Probable error.....	8.56	-----
D/P. E.....	.35	-----

In this table the inheritance of rusty-blotch resistance in the Mendelian ratio of three diseased to one disease-free plant is positively indicated, since the deviation is well within the limits of probable error. As immunity to rusty-blotch attack was completely dominant in  $F_1$ , the appearance of fixed recessive plants fully susceptible to rusty blotch is indicated. In the  $F_2$  plants this segregation could only be indicated by intensity of attack, which is not positively reliable (9). Further tests of these  $F_2$  plants were made, therefore, in the  $F_3$  generation in plant-to-row tests. The results are presented in Table 2.

TABLE 2.—Segregation of 275  $F_3$  families obtained in a cross between Chevalier, a variety immune to rusty blotch, and Abyssinian, a susceptible variety; plant-to-row tests in 8-foot rows

Item	Number of $F_3$ families immune	Number of $F_3$ families susceptible	Number of $F_3$ families containing both immune and diseased plants
Observed.....	94.00	68.00	113.00
Expected (1:1:2).....	68.75	68.75	137.50
Deviation.....	25.25	- .75	-24.50
Probable error.....	4.84	(?)	(?)
D/P. E.....	5.21	.15	5.00

As was expected, complete susceptibility appeared in the  $F_3$  families as a pure recessive in an almost perfect 3:1 ratio, all plants in each of these rows being attacked by rusty blotch. In the group of immune families the deviation from the theoretical or expected ratio was great (+25). This wide deviation may be accounted for by the difficulties in detecting slight attacks of rusty blotch in the presence of heavy mildew infection and the occurrence of small lesions of spot blotch. The small number in the group of heterozygous families (-25) is accounted for by the misplacement of this number in the immune group.

#### INHERITANCE OF SPECIES CHARACTERS IN A CROSS BETWEEN HORDEUM DISTICHON AND H. DEFICIENS

The barley cross between Chevalier and Abyssinian made to study the inheritance of resistance to rusty blotch involved two species, according to Harlan's (6) classification. Chevalier, included in the



species *Hordeum distichon*, is a two-rowed barley with lateral florets possessing glumes of usual size reduced, blunt, but easily discernible lemma and palea, small but complete anthers, reduced ovary, and stigmas with infertile ovules. Abyssinian is also a two-rowed variety included in the species *Hordeum deficiens* in which the glumes alone of the lateral florets are normal in size. The lemma is much reduced and pointed, the palea is usually indiscernible, and only rudimentary traces of the sexual organs remain.

The  $F_1$  plants in the cross Chevalier  $\times$  Abyssinian and the reciprocals all gave the characteristic form of the *Hordeum distichon* parent except for the lemma, which was sharply pointed instead of blunt. This condition has been noted by Biffen (3), Engledow (4), and Gillis (5). The  $F_2$  plants segregated for species inheritance into the groups presented in Table 3.

TABLE 3.—Summary of the  $F_2$  segregation of 860 plants obtained in cross of *Hordeum distichon* (Chevalier)  $\times$  *H. deficiens* (Abyssinian)

Item	Class 1, <i>H. distichon</i> type	Class 2, inter- mediate type	Class 3 <i>H. deficiens</i> type
Observed.....	226 00	413. 00	221. 00
Expected ratio, 1:2:1.....	215 00	430. 00	215. 00
Deviation.....	+11. 00	-17. 00	+6. 00
Probable error.....	8. 56	8. 56	8. 56
D/P. E.....	1. 28	1. 98	. 70

Little difficulty was encountered in placing the plants belonging to the parent groups, but the heterozygous plants showed a wide range of variation in the lateral florets. The subtending, or outer, glumes did not vary, but the lemma and palea varied not only in size but in shape. (Figs. 3 and 4.) Frequently heterozygous plants of the *Hordeum distichon* type produced greatly enlarged lemmas as compared with those normal for the *H. distichon* parent. Among the heterozygous plants resembling the *H. deficiens* parent many carried lemmas with blunt and rounded points instead of sharp pointed ones. The palea was frequently absent, but when present varied in size and shape. The sexual organs of these heterozygotes ranged from an entire absence of anthers and ovary to fully developed ones, but in no case were the lateral florets fertile. Fertility in all median florets was perfect in all phenotypic groups, no fertility being observed, as might possibly be expected, in species crosses.

The segregations fell into three classes, the two homozygous for the two parent species and the third or heterozygous group.

The Chevalier, or *Hordeum distichon* type, being recessive as shown in the  $F_1$  generation, was readily separated and closely approximated the expected ratio. Greater difficulty was encountered in separating the homozygous plants of the Abyssinian (*H. deficiens*) type. This, in a measure, was due to the presence of small anthers in the heterozygous types (fig. 5) which were quite easily overlooked in the examination of so great a number of plants. It is evident, however, that a 1:2:1 Mendelian ratio showing a single-factor difference exists between the two species (9).

In order to verify the ratio obtained in the  $F_2$  generation, seeds from these plants were sown during the following season in 8-foot rows. The segregation of the resulting families appears in Table 4.



FIG. 3.—Parent plants and  $F_2$  progeny of a cross between Chevalier, immune to rusty blotch, and Abyssinian, a susceptible variety: A, Chevalier (*H. distichon*) parent, immune to rusty blotch; B, Abyssinian (*H. deficiens*) parent, susceptible to rusty blotch; C, Abyssinian type ( $F_2$ ), susceptible, spike long and lax like Chevalier; D, Abyssinian type ( $F_2$ ), susceptible, long pointed lemmas; E, Chevalier type ( $F_2$ ), susceptible, lateral florets much enlarged; F, Abyssinian type ( $F_2$ ), no rusty blotch, lateral florets heterozygous type

TABLE 4.—Segregation in 275  $F_3$  families according to species inheritance of a cross between *Hordeum distichon* and *H. deficiens* and the reciprocal

Item	Number of $F_3$ families of <i>H. distichon</i> type	Number of $F_3$ families of <i>H. deficiens</i> type	Number of $F_3$ families of heterozygous type
Observed.....	65.00	77.00	133.00
Expected (1:2:1).....	68.75	68.75	137.50
Deviation.....	-3.75	+8.25	-4.50
Probable error.....	4.84	4.84	4.84
D/P. E.....	.77	1.70	.93

The  $F_3$  families segregated in the 1:2:1 ratio as shown in the  $F_2$  plants. Those plants which were homozygous for the *Hordeum distichon* and the *H. deficiens* groups gave rise to homozygous families in  $F_3$ , while the heterozygous plants grouped into homozygous groups for each of the parent types and into heterozygous groups, as was to be expected. The same variability in size and shape of lemmas, presence and absence of palea, occurred as were observed in the  $F_2$  plants.

CORRELATION BETWEEN RUSTY-BLOTCH ATTACK AND SPECIES INHERITANCE

In the  $F_1$  plants of the cross Chevalier (*Hordeum distichon*, immune to rusty blotch)  $\times$  Abyssinian (*H. deficiens*, susceptible) and reciprocals, immunity to rusty blotch and the *H. distichon* form appeared dominant. In the  $F_2$  generation when the homozygous *H. deficiens*, the homozygous *H. distichon*, and the intermediate plants were each segregated in regard to their resistance or susceptibility to rusty blotch the distribution appears as shown in Table 5.

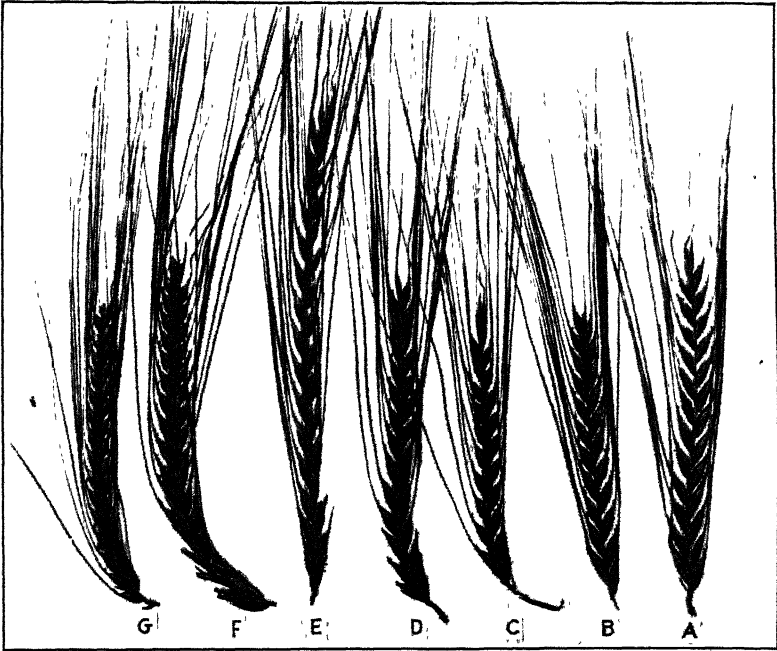


FIG. 4.— $F_2$  plants from a cross between Chevalier and Abyssinian: A, Spike lax like Chevalier but lemmas like Abyssinian, only larger and more pointed; B, spike dense and lateral florets like Abyssinian; C, spike dense like Abyssinian but lateral florets like Chevalier; D, spike dense like Abyssinian but lateral florets and lemmas like Chevalier; E, spike lax like Chevalier but florets intermediate in size and shape; F, spike with intermediate density but florets like Chevalier only much enlarged and infertile; G, spike dense like Abyssinian but florets like Chevalier, enlarged but infertile

TABLE 5.—Rusty blotch attack in the  $F_2$  plants of the homozygous and heterozygous groups

Item	Class 1, <i>H. distichon</i> , homozygous			Class 2, intermediate or heterozygous			Class 3, <i>H. deficiens</i> , homozygous		
	Number of plants not attacked	Number of plants attacked	Total	Number of plants not attacked	Number of plants attacked	Total	Number of plants not attacked	Number of plants attacked	Total
Observed.....	173.000	53.000	226.00	309.00	86.00	395.00	146.000	75.000	221.00
Expected (1:2:1) (3:1).....	157.875	52.625	210.50	315.75	105.25	421.00	157.875	52.625	210.50
Deviation.....	+15.125	+.375	+15.50	−6.75	−19.25	−26.00	−11.875	+22.375	+10.50
Probable error.....	4.390	4.390	8.47	5.80	5.80	8.47	4.340	4.340	8.47
D/P. E.....	3.400	.080	1.83	1.16	3.30	3.07	2.700	5.20	1.24

The results presented in Table 5 indicate that both the *Hordeum distichon*-*H. deficiens* contrast and resistance versus susceptibility to rusty blotch conform to the Mendelian law of segregation, inasmuch as the distribution of *H. distichon*, heterozygous, and *H. deficiens*

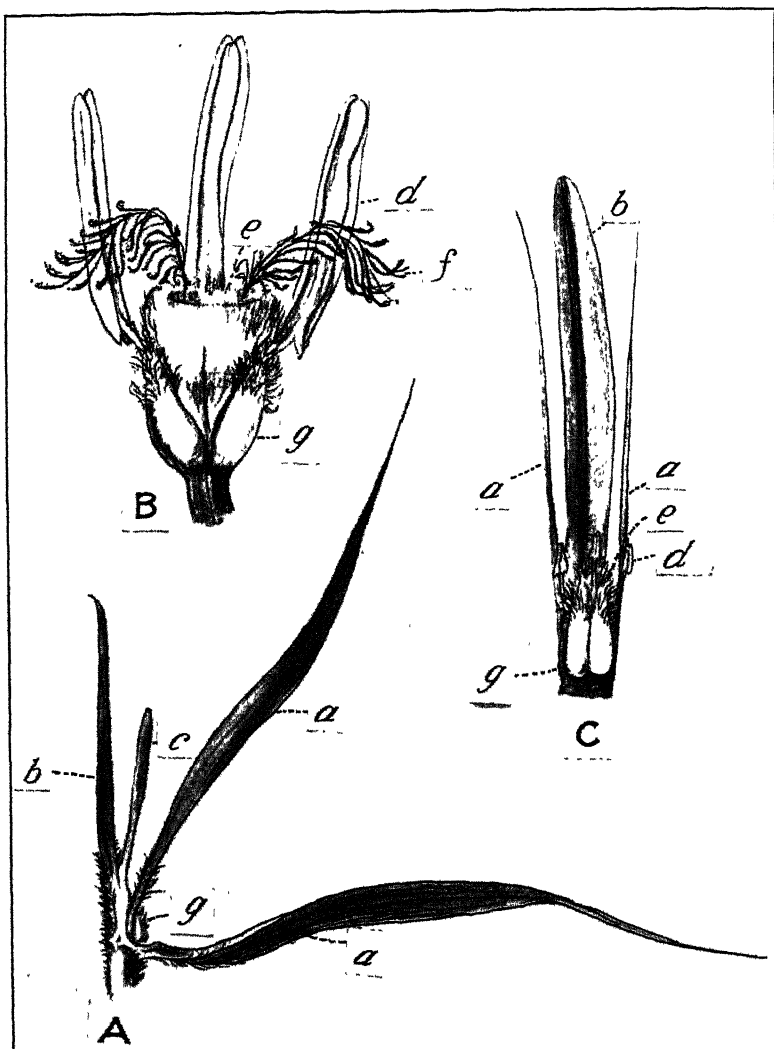


FIG. 5.—A, lateral floret of Abyssinian (*H. deficiens*); B, median floret of Chevalier (*H. distichon*); C, lateral floret of Chevalier: a, Glume, b, lemma; c, palea, d, anther; e, ovary; f, stigma; g, lodicule

plants closely approximates the 1:2:1 ratio, while in each of these 3 classes is found an approximation to the 3:1 ratio of resistant to susceptible plants. The theoretical distribution to be expected on the basis of segregation and independent assortment of two pairs of factors with incomplete dominance in one pair, using the conventional symbols *Aa* for *distichon* v. *deficiens*, and *Bb* for resistance v. susceptibility, would be 3*AB*: 1*Ab*: 6*AaB*: 2*Aab*: 3*aB*: 1*ab*. When

the observed distribution is compared with the theoretical numbers expected in case of such factorial relations, the deviations, as shown in Table 5, are rather wide, giving a value for D/E of more than 3 in 4 of the classes and of more than 5 in 1 class. Although considerable difficulty was experienced in classifying the *deficiens* plants which were attacked on account of the presence of other closely similar diseases, this does not explain the wide discrepancy in the case of *distichon* plants not attacked. It seemed worth while, therefore, to examine the observed distribution for the existence of genetic correlation. The data are assembled for comparison with the theoretical 9:3:3:1 distribution for two pairs of characters by combining the *H. distichon* and intermediate groups as is shown in Table 6.

TABLE 6.—The data from Table 5 revised for comparison with the theoretical dihybrid distribution

Item	Classes 1 and 2		Class 3	
	Not attacked	Attacked	Not attacked	Attacked
Observed.....	482 000	139 000	146 000	75 000
Expected (9:3:3:1).....	473 625	157 875	157 875	32 625
Deviation.....	+8 375	-18.875	-11.875	+22 375

The wide deviation from the theoretical 9:3:3:1 distribution shown in Table 6 at once suggests linkage between the two pairs of factors concerned. Applying the method of Babcock and Clausen (1, p. 132-142), the linkage value is found to be approximately 35. When, however, the observed distribution is compared with the theoretical distribution into six classes on the basis of 35 per cent of recombination in  $F_2$ , as shown in Table 7, the deviations are found to be just about as serious as those obtained when it was compared with the distribution expected on the basis of independent inheritance of the two pairs of factors. From the present data, therefore, it is impossible to decide whether or not there is genetic correlation between the *Hordeum distichon*-*H. deficiens* contrast and resistance v. susceptibility to rusty blotch. Until further data are available, however, it may be assumed that such correlation does not exist.

TABLE 7.—Observed distribution in  $F_2$  from the cross *Hordeum distichon* resistant  $\times$  *H. deficiens* susceptible, as compared with theoretical distributions in case of independent inheritance and linkage with 35 per cent of recombinations in  $F_2$

Phenotypic classes	Independent assortment	Observed distribution	35 per cent recombinations AB $\times$ ab	Phenotypic classes	Independent assortment	Observed distribution	35 per cent recombinations AB $\times$ ab
AB.....	157.875	173	184.7	Aab.....	105.250	86	95.7
Ab.....	52.625	53	25.8	aB.....	157.875	146	121.5
AaB.....	315.750	309	325.2	ab.....	52.625	75	88.9

A test of  $F_3$  families grown in 1927 from  $F_2$  plants was made. The observations are recorded in Table 8.

TABLE 8.—Distribution in  $F_3$  families of rusty-blotch resistance and susceptibility in homozygous and heterozygous groups

Item	$F_3$ families of homozygous <i>H. distichon</i> group				$F_3$ families of heterozygous group				$F_3$ families of homozygous <i>H. deficiens</i> group			
	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total	Number of rows total	Number of rows free of rusty blotch	Number of rows attacked by rusty blotch	Total
Observed.....	80.00	28.00	52.0	80.00	138.00	31.00	107.0	138.00	62.00	16.00	46.0	62.00
Expected.....	70.00	20.00	60.0	70.00	140.00	34.50	105.5	140.00	70.00	15.50	46.5	70.00
Deviation.....	+10.00	+8.00	-8	+10.00	-2.00	-3.50	+3.5	-2.00	-8.00	+0.50	-1.5	-8.00
Probable error.....	4.89	2.61	-----	4.89	4.89	3.43	-----	5.64	4.89	2.30	-----	4.89
D/P. E.....	2.04	3.06	-----	2.04	.41	1.02	-----	.35	1.63	.22	-----	1.63

The expected 1:2:1 distribution for *Hordeum distichon* intermediate and *H. deficiens* is realized. The ratios of resistant to susceptible plants in these  $F_3$  families are seriously disturbed by the very evident difficulty in detecting rusty-blotch attack when the lesions are small or obscured by mildew.

Undoubtedly some plants were genetically susceptible to rusty blotch but escaped attack. For these reasons rusty-blotch families observed are undoubtedly in excess of those genetically immune to attack. The homozygous *Hordeum deficiens* group has suffered a reduction, while the homozygous *H. distichon* group has been increased because of difficulties arising in the shape of the lemma, especially its point.

#### DISCUSSION

Species crosses usually result in partial or complete sterility in the offspring. Such behavior in hybrids may defeat the breeder working to create disease-resistant varieties. The two species involved in these breeding investigations gave, when crossed, complete fertility of the median florets. (Figs. 3 and 4.) The lateral florets are infertile, as both are two-rowed barleys (*Hordeum distichon* and *H. deficiens*). This finding is contrary to the findings of Biffen (3), Engledow (4), Hor (7), and Gillis (5), who encountered sterility to a greater or less degree in species crosses with barley, especially when six-rowed and two-rowed species were involved. Stimulation causing increase in length and width of lemma and palea in lateral florets when the two-rowed species *H. distichon* and *H. deficiens* were crossed has been recorded by Biffen (3), Engledow (4), Hor (7), and Gillis (5). Biffen found a single-factor difference to exist between these crosses, but the goodness of fit on which he based his 1:2:1 ratio was not close, 144 (28 per cent):259 (50.3 per cent):112 (21.7 per cent).

Agronomic characters for resistance to lodging and early maturity were studied, but no extensive counts were made. It appears from the evidence gathered that resistance to lodging (a character possessed by the Abyssinian parent) is dominant, as indicated by the  $F_1$  and  $F_2$  generations. Erect *v.* nodding heads—characters appearing in the Abyssinian and Chevalier parents, respectively—indicate that the erect head character is dominant in  $F_1$  and  $F_2$  generations. Early maturity was likewise found to be a dominant character.

## SUMMARY

In crosses between Chevalier, an immune variety of barley, and Abyssinian, a susceptible variety, and their reciprocals, no rusty-blotch attack occurred in the  $F_1$  plants.

In the  $F_2$  generation the segregation occurred in the proportion of three nonattacked plants to one attacked, or in a 3:1 ratio, indicating a single factor difference for rusty-blotch resistance.  $F_3$  families from the  $F_2$  plants confirmed this ratio.

The two species involved in these crosses (*Hordeum distichon* and *H. deficiens*) gave in  $F_1$  *H. deficiens* like plants, which gave rise in the  $F_2$  generation to plants homozygous for both *H. distichon* and *H. deficiens* and intermediate in a Mendelian ratio of 1:2:1. This ratio was confirmed by the  $F_3$  families from these  $F_2$  plants.

No infertility was observed as a result of species crossing.

No decided evidence of linkage was found.

## LITERATURE CITED

- (1) BABCOCK, E. B., and CLAUSEN, R. E.  
1927. GENETICS IN RELATION TO AGRICULTURE. Ed. 2, rev., 673 p., illus.  
New York and London, McGraw-Hill Book Company, Inc.
- (2) BEAVEN, E. S.  
1902. VARIETIES OF BARLEY. Jour. Fed. Inst. Brewing 8: 542-594, illus.
- (3) BIFFEN, R. H.  
1905. THE INHERITANCE OF STERILITY IN THE BARLEYS. Jour. Agr. Sci. [England] 1: [250]-257, illus.
- (4) ENGLEDDOW, F. L.  
1920. INHERITANCE IN BARLEY. I. THE LATERAL FLORETS AND THE RACHILLA. Jour. Genetics 10: [93]-108, illus.
- (5) GILLIS, M. C.  
1926. A GENETICAL STUDY OF THE FERTILITY OF THE LATERAL FLORETS OF THE BARLEY SPIKE. Jour. Agr. Research 32: 367-390, illus.
- (6) HARLAN, H. V.  
1918. THE IDENTIFICATION OF VARIETIES OF BARLEY. U. S. Dept. Agr. Bul. 622, 32 p., illus.
- (7) HOR, K. S.  
1922. A NEW VARIETY OF BARLEY WITH STRIKING CHARACTERISTICS. Science (n. s.) 55: 378.
- (8) KÖRNICKE, F.  
1885. DIE ARTEN UND VARIETÄTEN DES GETREIDES. In Körnicke, F., and Werner, H., Handbuch des Getreidebaues, Bd. 1, p. 1-378, illus. Berlin.
- (9) MACKIE, W. W.  
1926. INHERITANCE OF RESISTANCE TO HELMINTHOSPORIUM CALIFORNICUM IN A CROSS BETWEEN CHEVALIER BARLEY, A RESISTANT VARIETY, AND ABYSSINIAN, A SUSCEPTIBLE VARIETY. (Abstract) Phytopathology 16: 764.
- (10) ——— and PAXTON, G. E.  
1923. A NEW DISEASE OF CULTIVATED BARLEY IN CALIFORNIA CAUSED BY HELMINTHOSPORIUM CALIFORNICUM, N. SP. Phytopathology 13: 562.
- (11) WIGGANS, R. G.  
1921. A CLASSIFICATION OF THE CULTIVATED VARIETIES OF BARLEY. N. Y. (Cornell) Agr. Expt. Sta. Mem. 46, p. 365-456, illus.





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## EXPERIMENTS WITH CLASSES OF STOCK SUITABLE FOR FOREST PLANTING IN THE NORTHERN ROCKY MOUNTAINS<sup>1 2</sup>

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### INTRODUCTION

Except in unusually moist years the survival of planted stock in the northern Rocky Mountains, even on favorable slopes, has not been satisfactory. On good and poor sites alike, the principal cause of the unusual degree of mortality has been the combination of dry soil and dry winds during the hot days of July and August. The results have made it clear that the most important consideration in forest planting in this region is not the choice of a favorable site for planting, but the kinds of planting stock that are best suited to survive the unusually dry conditions, regardless of site.

Records of the United States Weather Bureau taken at Haugan, Mont., near the site of some of the experiments to be described here, indicate that the normal rainfall at that point during June is less than 2 inches, during July about 1 inch, and in August less than 1 inch (2).<sup>4</sup> At the same time low humidity, high temperatures, and wind during these months increase evaporation and thus increase the odds against which the planted trees must contend (4).

If it were possible to predict with certainty the occurrence of dry years, forest planting in this region could very profitably be confined to moist years, and in this way, with ordinary care, the immediate needs for planting could be met and a high degree of success would be insured. Large investments could be made in planting projects with reasonable assurance of satisfactory survival regardless of the quality of stock. Such reliable forecasts of weather conditions are, however, nowhere available as yet. The only factor directly under control is the class of stock to be grown for planting.

It is the purpose of this article to present the results of experiments in selection of planting stock that may be advantageously applied any year in any of the planting work in this region. The experiments, based on standard practices, were planned to measure the effects of length of roots, age, and size of trees on the survival of the stock in time of drought.

### MATERIALS AND METHODS USED

The two tree species used in these experiments were the western white pine (*Pinus monticola*) and the western yellow pine (*P. ponderosa*). The planting sites were chosen only as typical of the class

<sup>1</sup> Received for publication May 9, 1928; issued August, 1928.

<sup>2</sup> Planting by the United States Forest Service in this region is confined at present to that part of the western white pine (*Pinus monticola*) belt in the St. Joe, Coeur d'Alene, Cabinet, Pend Oreille, and Lolo National Forests. Only land which was recently burned over twice is planted, because the areas burned only once usually are reseeded and the forest is satisfactorily reproduced by natural methods.

<sup>3</sup> The following men took part in the early experiments: D. R. Brewster, J. A. Larsen, E. C. Rogers, and P. C. Kitchen. The author made the later experiments.

<sup>4</sup> Reference is made by number (italic) to "Literature cited," p. 1000.

of land on which each species was being extensively planted. The white pine land ranged in elevation from 2,000 to 4,000 feet on northerly slopes and 4,000 to 5,000 feet on westerly and southerly slopes, but no southerly slopes were planted with western white pine unless the soil proved to be very favorable. Western yellow pine plantations were on elevations ranging from 2,000 to 4,000 feet on all slopes. Northerly slopes were, however, invariably planted with white pine rather than yellow pine.

The planting stock was lifted by means of spading forks, except when unusually long (10-inch) roots were desired and a spade was needed. The effort was made to handle uniformly all of the trees in any one experiment. Culling and heeling in followed the usual nursery practice. All of the grading, root pruning, sorting, etc., was done in the shade of a building and with the constant use of wet burlap to prevent drying of roots.

In all experiments, except as otherwise mentioned, planting was by the "slit" method, illustrated in Figure 1, used by the administrative planting crews of the Forest Service in the region. The more careful methods of planting, such as the cone method, may be ideal where cost is a minor consideration, but the slit method has here proved to be most efficient; it has yielded the largest number of living trees per dollar invested, and has shown no evidence of subjecting the trees to root rot or windfall in later life.

Season of planting was given scant consideration. A few experiments and several years of practice indicate that no great difference is to be expected between spring and fall plantings for an average year. Causes of loss of trees may differ somewhat for the two seasons and from one year to another, but the final effect on survival has usually been about equal for spring and fall work. The cold nights and warm days of an Indian summer following fall planting are apparently detrimental. If such weather does not occur, there may be an advantage in fall planting over that of the following spring, but this possibility seems to have no practical value in view of the present impossibility of forecasting such conditions.

As a rule, all trees planted in the field for experimental purposes were individually marked with stakes bearing symbols which designated the test. Sometimes individual serial numbers were used in order that a separate record might be kept for each plant. Lath stakes with tips painted white were used as markers when it was desired to observe the plants closely for three years or more. Otherwise the smaller and less durable, but more easily handled, manufactured pot labels or garden stakes were used to economize labor.

Spacing in plantations was not important in connection with studies that were not to be followed to the stage when crowns close. Hence, the usual method of spacing in plantations was modified to facilitate the taking of the frequent records needed. The different test lots to be compared were usually set in alternate rows, but sometimes, in order to mix them better, the two lots were set in the same row with plants alternating. The rows were of sufficient length to contain from 40 to 200 plants and ran approximately at right angles to contours. These arrangements, together with the use in each test of as uniform a slope as possible, tended to reduce experimental error due to local and unavoidable variations of site. An effort was made either to base each test lot on not less than 400 trees or to repeat the work during several seasons.

## LABORATORY WORK

Because of the undesirability of field-planting trees from which detailed anatomical measurements had been taken and which had thereby been exposed to injury from drying, sample lots were devoted

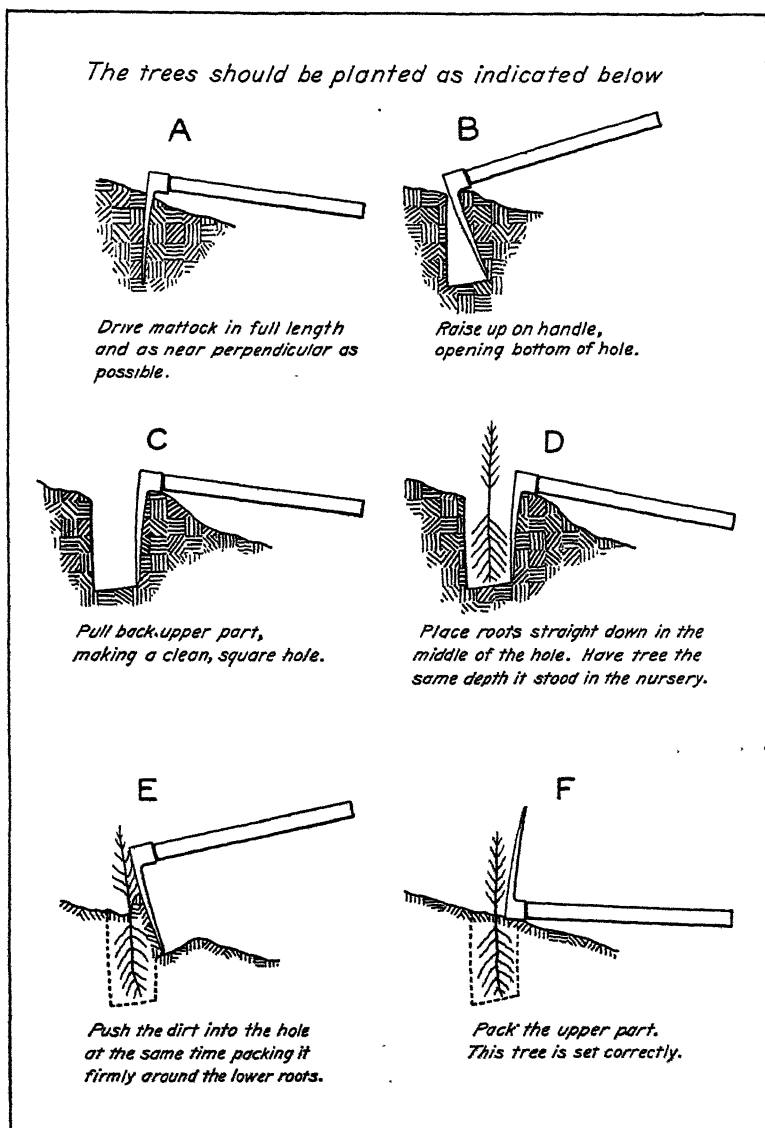


FIG. 1.—Diagram illustrating the proper use of the slit method of planting trees

exclusively to laboratory examination. These were selected mechanically in order to eliminate the factor of personal judgment. For example, if it was desired to plant 400 and to use 100 in the laboratory, then in counting out the 500, every fifth plant was reserved for

study. In the laboratory every plant was tagged with a serial number and, ordinarily, the following measurements and counts were made:

(1) Diameter at the ground line in millimeters and tenths by means of a micrometer caliper.

(2) Length of stem from ground line to tip of terminal bud in inches and tenths.

(3) Number of primary lateral rootlets in two-length classes: (a) 0.5 to 2 inches, and (b) over 2 inches long.

(4) Number of secondary lateral rootlets divided into two classes on the same basis. By primary rootlet is meant a branch from the central or tap root, and all branches from the primary rootlets are called secondary.

(5) The length of one needle of average appearance taken from the center of the crown, in inches and tenths.

All measurements were then averaged, and from each lot a few plants having measurements falling close to those of the average tree were selected from the data sheets. The trees thus chosen as typical of stock planted were identified by their tag numbers and were photographed against a translucent screen, using only transmitted light, to obtain satisfactory silhouettes. These plants were severed at the ground line. In some tests the trees were cut just below the crown and also just above the root collar, the intervening section of bare stem being discarded because it functioned only in mechanical support and conduction. After separation, the masses of tops and roots were dried in a water-jacketed oven until no further loss of weight occurred. The averages resulting from the weights of this dried material were useful in judging relative plant development and the balance between top and roots.

#### EXAMINATION OF PLANTATIONS

In taking records of the plantations an effort was made to make the first examination three weeks or a month after planting in order to observe the early unthriftiness or death due to the shock that plants receive from being moved. Thereafter, during the first season, at least three examinations were made, not oftener than once a month. This was necessary in order to detect the trends of changes leading to the final condition of the trees in the fall. During the second season spring and fall examinations were usually sufficient.

Plants were classified merely as thrifty, unthrifty, and dead, with a notation of any fresh evidences of causes of unthriftiness or death. There was difficulty in fixing a dividing line between thriftiness and unthriftiness, and in the recent experiments a tree was not classed as unthrifty unless this condition was so well marked that it was believed the chances were even that the plant would not recover. Possibly the word "failing" would be more appropriate than "unthrifty." Many trees, suffering from the shock of planting and the first effects of drought, exhibited a partial browning of the needles formed the previous season which gave the plant an unthrifty appearance in the popular sense. Experience indicates that a large number of such plants usually recover, and, therefore, the present policy is to be conservative in calling trees unthrifty. This gives more meaning to a plant class which, at best, depends for its true significance on the accuracy and consistency of personal judgment. With very few exceptions, all test and control lots of trees were planted by the same man, and all records in any series were made by the same person.

## TESTS OF AGE CLASSES

Besides showing how old trees are, the term "age class," as applied to forest nursery stock, refers to differences in time of transplanting in the nursery and hence indirectly indicates differences in plant development. Thus the age class 2-0 refers to seedlings which have been in the seed beds for two growing seasons and not transplanted; 1-2 refers to 3-year-old transplants that spent only their first year in the seed beds; and 1-1-1 refers to stock that is 3 years old and was transplanted each year in the nursery. For any given species of tree it is these differences in development that influence the quality of

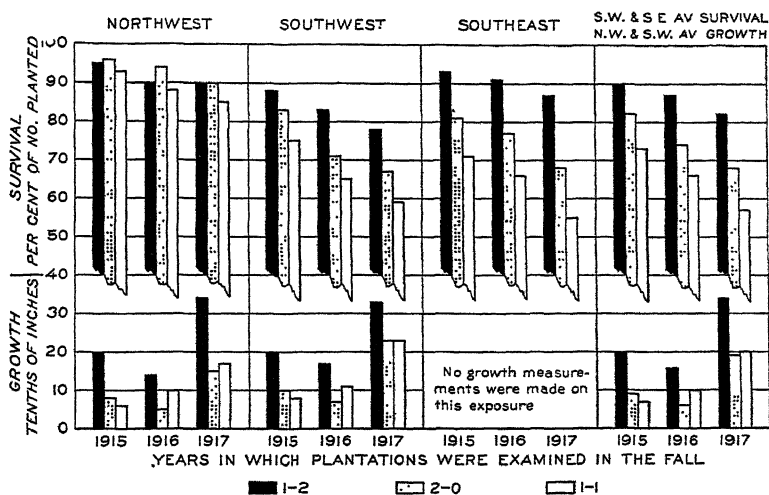


FIG. 2.—Survival and growth of western yellow pine: 7,200 trees of different age classes planted in the spring of 1915 near Wallace, Idaho

stock. In the experiments, age classes were tested in field plantations in order to guide the nursery work.

## WESTERN YELLOW PINE

The earliest satisfactory experiments with western yellow pine age classes were started in the spring of 1915 on the Placer Creek drainage, near Wallace, Idaho, on an area severely denuded by the Idaho conflagration of 1910, and typical of the doubly burned-over land in need of reforestation in this region. About 7,200 trees divided equally among three age classes were planted on slopes of three different aspects. All members of the planting crew planted an equal number of trees in each test lot, thus excluding the effect of differences in quality of work on the comparison of later behavior of the trees. Growth measurements were made only on thrifty trees. Although the amount of rainfall during the summer of 1915 was about 2.5 inches above normal for the locality, most of the deaths of trees in the plantations could be attributed to no other cause than drought, and the data therefore indicate directly the relative drought-resisting powers of the several age classes, especially on the more severe sites.

The results of observations of growth and survival during three years are recorded graphically in Figure 2. It may be seen from the

diagram that, on the northwest slope, where the percentage of survival was highest for all age classes, the differences in survival were not great enough to justify any comparison of the classes of stock, but on the two southerly slopes, more definite relationships were apparent. Clearly, the 1-2 stock was best able to survive planting, the 2-0 was intermediate, and the 1-1 stock was least well fitted to survive. Differences in growth between the 2-0 and 1-1 stock were negligible, but the 1-2 stock grew fastest from the start, on all slopes.

With vast areas of forest land lying idle, it is necessary first of all to make each dollar produce as many living trees as possible. Therefore, these age classes were also compared on the basis of cost of survival, or cost of planting per living tree. The cost per thousand survivors of each class was computed by dividing the total cost of planting a thousand trees by the percentage of survival attained and multiplying by 100.

The first cost of planting, including the production of stock, is usually greatest for large transplants. In this case, the 1-2 cost most, the 1-1 next, and the 2-0 least. When the cost of survival was computed, the age classes in the experiment compared as follows: On the favorable northwest slope, the 2-0 stock was most effective, the 1-1 next, and the 1-2 least desirable. On the less favorable southwest and southeast slopes, 1-2 was superior, 2-0 intermediate, and 1-1 inferior. The 1-1 stock made an especially poor showing on the southeast slope from this point of view. Considering the theoretical average cost of survival on all three slopes after three years, the 1-2 stock was most economical, with the 2-0 stock a close second. The survival of 2-0 stock was but 2 per cent more expensive than the 1-2, whereas the 1-1 stock was 23 per cent more expensive.

Very little 2-1 stock has been grown at Savenac. As a general rule, it has been observed in practice that the so-called shock of transplanting or setback in growth which follows the setting of a seedling in a new location persists throughout the following year. In other words, during the first year after transplanting the benefits usually appear to be entirely offset by loss of growth. This condition can be more easily understood from the fact that the 2-1 stock at Savenac Nursery develops quite irregularly, resulting in inferior development of about half of the plants. Culling out about half of the number makes the remainder expensive.

Correspondingly little has been done with 2-1 western yellow pine stock in the way of experimental field planting. The results of two early tests are conflicting. In the fall of 1912 a field test of 2-1 stock was installed at Priest River, Idaho, together with 1-2 and other stock. A year later 78 per cent of the 2-1 were alive and 79 per cent of the 1-2, but the costs per thousand living trees were \$23.28 for the 2-1 and \$18.94 for the 1-2. In the spring of 1918 another test was made at Haugan, Mont., and was watched during three years. This time the 2-1 led decisively. Its survival was 61 per cent, as compared to 43 per cent for 1-2 the first year, and 54 per cent as against 39 per cent for 1-2 the third year in the field. A comparison of the cost of survival showed the 2-1 stock to be \$4.73 cheaper per thousand third-year survivors.

In May, 1923, a test of 2-1, 1-2, and 2-0 western yellow pine was made on a southeast slope of the 1910 burn near Haugan, Mont. The site bore numerous ceanothus bushes and some grass, being typi-

cal of much of the nonforested land of the region. The trees were lifted from the beds at Savenac Nursery on May 1. Not more than 3 per cent were rejected as unsuitable for field use and most of these were culled because of broken roots. In bunches of 50, all plants were root pruned at a point 6.5 inches from the ground line, removing about 3 inches from 2-0 roots, 3.5 inches from the 1-2, and 2 inches from the 2-1. About 50 trees from each lot were set aside for use in the laboratory, and 200 of each of the age classes were planted.

The trees were examined three times during 1923 and twice during 1924 in order to observe the causes of unthriftness and death. There could be no doubt that drought was the principal cause. No marked differences in the condition of the three lots of trees occurred in the first year until the soil became dry in September. Survival was then 92 per cent of the number planted for 2-1 stock, 90.5 per cent for 1-2, and 84.5 per cent for 2-0. Dry weather started early in the spring of 1924, and thus more deaths than usual occurred during the second growing season, but the age classes still maintained the same order of drought resistance. (See Table 1.)

TABLE 1.—*Comparison of the percentages of survival and top-root ratios of western yellow pine of three different age classes at end of second growing season* <sup>a</sup>

Age class	Alive	Dead	Thrifty	Unthrifty	Weight of plant in top
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
2-1 stock.....	81.8	18.2	80.3	1.5	64.0
1-2 stock.....	77.1	22.9	76.1	1.0	71.5
2-0 stock.....	72.9	27.1	69.9	3.0	74.4

<sup>a</sup> Condition in October, 1924, of plantations made in the spring of 1923 on a southeast slope of the 1910 burn near Haugan, Mont.

As is shown in Table 1, field survival increased in the same order in which the percentage of total plant weight in the tops decreased. In other words, survival increased as the top-root ratio decreased. The 2-1, which survived best, had the best balance, whereas the seedling 2-0 stock, which survived least well, had the poorest balance. As will be seen later, this relation between balance and field survival does not always hold true, but the tendency is unmistakable and it should be a help in judging the ability of stock to withstand dry conditions. The appearance of the stock used in the experiments is shown by photographs of representative trees in Figure 3.

These tests indicate that 2-1 stock may excel 1-2 stock in survival if the percentage of weight in the top is less; but, because of the necessity of heavy culling and the consequent higher cost of production of 2-1 stock, it is doubtful if it will ever successfully compete with 1-2 stock. The results agree with the earlier experiments at Wallace in indicating the superiority of 1-2 over 2-0 planting stock.

This evidence of superiority was further corroborated a year later in an experiment testing size classes and length of root. In the spring of 1924, using a uniform grade of medium-sized stock with root systems pruned to a length of 8 inches, 491 plants of 1-2 and 494 plants of 2-0 stock were set out on a dry, rocky, severe south slope. The results are given in Table 2. About one-sixth of the trees in each

lot were dead when first examined, and the two lots were in similar condition when the dry weather started. Mortality with the 2-0 then proceeded at a more rapid rate, especially in July, than with the 1-2 stock, and, although both plantations were virtually destroyed the destruction of the 1-2 was less complete.

TABLE 2.—Mortality rate of two age classes of western yellow pine in a plantation destroyed by drought <sup>a</sup>

Age class	Survival at different dates				
	May 26	June 26	July 26	Aug. 26	Nov. 7
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1-2 stock.....	83.9	73.1	23.8	10.6	7.4
2-0 stock.....	82.8	62.1	7.7	2.6	1.4

<sup>a</sup> Condition during 1924 on a south slope or the 1910 burn near Haugan, Mont.

The experiments with western yellow pine age classes agree in showing the superior survival of 1-2 over 2-0 stock. When it has a small top-root ratio, 2-1 stock may equal or excel 1-2 in the field; 1-1 is inferior to the other classes. In cost of survival the use of 2-0 stock is scarcely less economical than the use of 1-2 stock. As to other possible age classes it may be said that 1-year-old seedlings, are too small, and that the rather rapid growth of western yellow pine causes seedlings more than 2 years old or transplants more than 3 years old to be too large for economical handling in planting operations at the present time.

Korstian and Baker (3), working in the intermountain region obtained the best survival with 2-1 stock. With them the other age classes ranked as follows in descending order of survival: 2-0, 2-2 1-2, 3-0, and 1-1.

#### WESTERN WHITE PINE

In 1911, at the Priest River Experiment Station <sup>5</sup> in Idaho, a small clearing of about 1 acre was made in a 60-year-old stand of western white pine, Douglas fir (*Pseudotsuga taxifolia*), and western larch (*Larix occidentalis*) on a northeast slope. In the spring of 1915, 100 plants each of 2-0 and 1-2 western white pine were planted in the clearing. In 1921, 66 per cent of the 2-0 and 78 per cent of the 1-2 plants were alive. The 2-0 plants averaged 12 inches and the 1-2 plants 19 inches high.

Another experiment with western white pine age classes near Wallace, Idaho, in the spring of 1915, was combined with a study of aspects of planting sites. About 600 trees were planted in each test lot, or 12,000 trees in all. The details of this experiment have been reported in an earlier paper (8); it will be sufficient here to give the results of the work. Figure 4 shows the relative survival of five different age classes at the end of the third growing season after planting. This diagram of actual survival disregards the cost and the growth rates of the living trees. With cost and growth considered, it may be concluded that although in survival of planted trees and in height growth 2-2 western white pine transplants are

<sup>5</sup> Now a field station of the Northern Rocky Mountain Forest Experiment Station.



at the top, with 1-2, 2-1, 2-0, and 1-1 following in descending order, the cost of survival definitely favors 2-0 seedlings on moderate sites, and 2-2 or 1-2 only on the more severe sites.

No further work of this kind was attempted until the fall of 1922. That year dry weather continued so late into the fall that very little planting could be done on the sites selected. On October 27, 400 plants each of 1-2 and 3-0 age classes were planted on a north-west slope of the 1910 burn near Haugan. At the close of the following season the 1-2 stock showed 4 per cent higher survival and 12 per cent more thrifty plants than the 3-0. The growing season had been moist until September, when a deficiency of rainfall caused plants to suffer from drought. Had the summer as well as the fall been dry, the difference in survival would probably have been greater, because the transplants (1-2) had over 10 per cent more of their total weight in their root systems than had the seedlings (3-0). By the end of another growing season the transplants had increased their lead by an additional 4 per cent in survival.

The next test, of 1-2, 3-0, and 2-0 western white pine, was started in the fall of 1924, the trees being lifted from the nursery during the last week of September. From the 2-0 stock 3 per cent and from the 3-0 and 1-2 stock 1 per cent of the trees were culled out because of extremely poor development or injury in lifting. Uniformity in the lot of trees was increased by culling out both the largest and smallest individuals until only about half of the original number remained. All roots extending more than 8 inches from the ground line were pruned off at the 8-inch point. Average measurements of

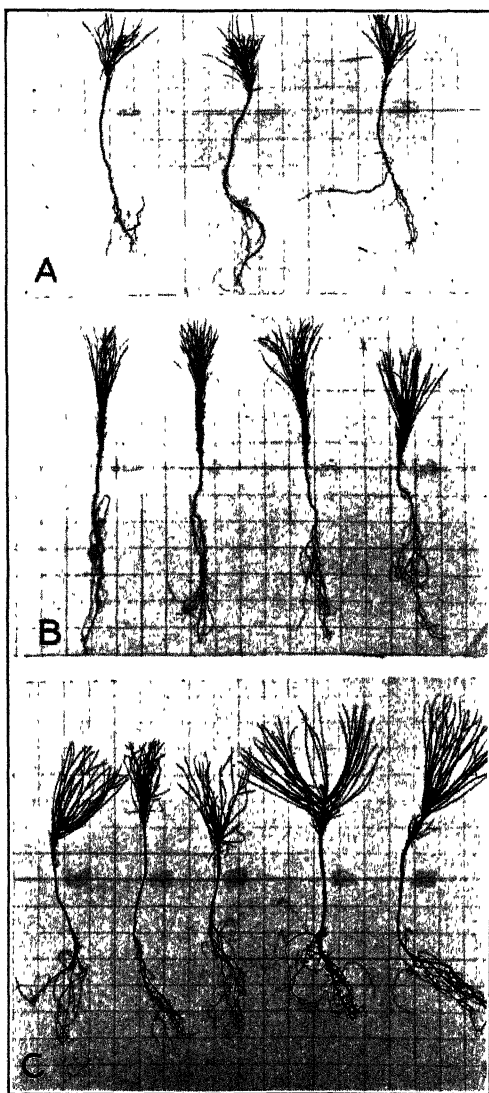


FIG. 3.—Western yellow pine age classes, 1923: A, 2-1 stock; B, 2-0 stock; C, 1-2 stock

these trees are given in Table 3, and the appearance of representative trees is shown in Figure 5. Four hundred trees of each of these age classes were planted in October, 1924.

TABLE 3.—Anatomical characteristics of western white pine stock planted in the fall of 1924, as determined by average measurements <sup>a</sup>

Age class	Stem length	Length of needles	Diameter at ground line	Lateral rootlets		Oven-dry weight	Weight in top	Weight in root
				0.5-2 inches	Over 2 inches			
	Inches	Inches	Milli-meters	Number	Number	Grams	Per cent	Per cent
1-2 stock	2.4	1.4	2.1	22.2	12.3	1.0	52	48
3-0 stock	4.1	1.5	2.5	20.1	12.8	1.6	67	33
2-0 stock	2.6	1.5	2.0	17.3	7.1	.7	61	39

<sup>a</sup> Basis: Weight measurements, 55 trees; other measurements, 60 trees.

In the following winter, during a period of exceptionally severe weather when the trees were but partly covered with snow, the tops appeared to be seriously injured. However, detailed observation failed to indicate any relation between the relative drought hardness

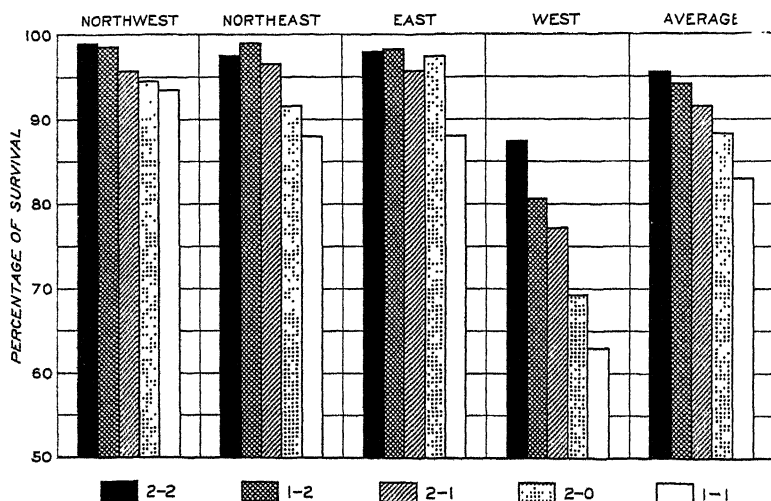


FIG. 4.—Survival of western white pine age classes: 12,000 trees three years after planting in the spring of 1915 near Wallace, Idaho

of the different age classes and either the numbers of buds remaining dormant or the extent of injury to foliage. During the following growing season the winter injury was seen to be less serious than was thought at first; adventitious buds formed readily, and, although the growth of several plants was checked, survival did not appear to be affected. At the end of the season the condition of the plantations was as shown in Table 4.

TABLE 4.—Condition in September, 1925, of western white pine age classes planted in the fall of 1924, on a northeast aspect near Haugan, Mont.

Age class	Alive	Dead	Thrifty	Unthrifty
	Per cent	Per cent	Per cent	Per cent
1-2 stock	85.7	14.3	83.7	2.0
3-0 stock	82.7	17.3	78.2	4.5
2-0 stock	72.0	28.0	70.7	1.3

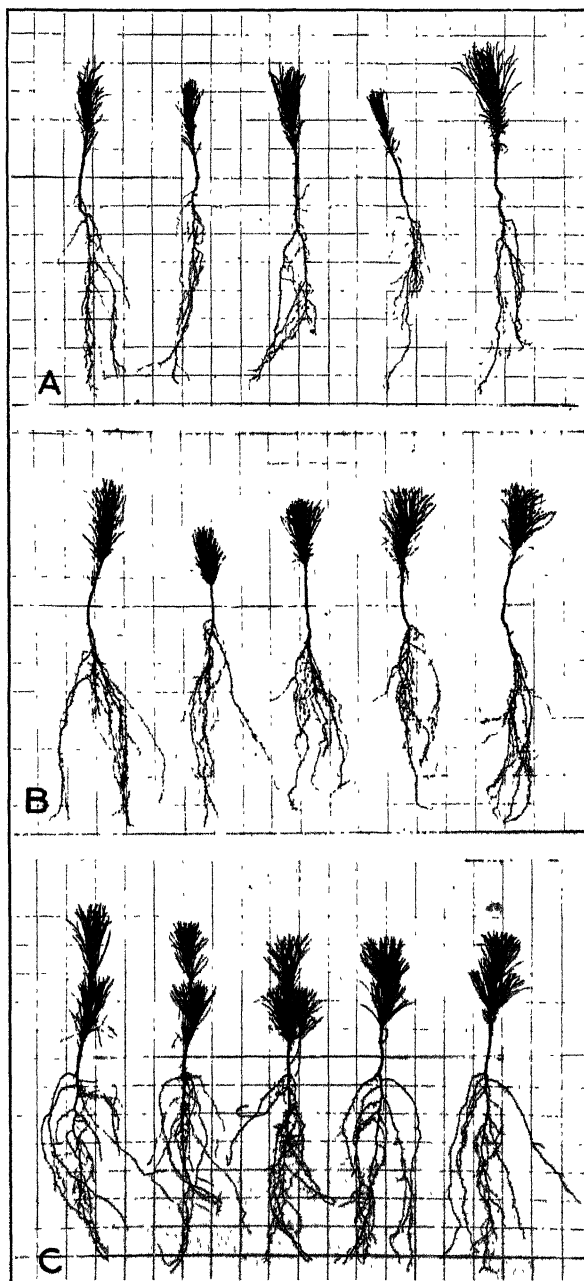


FIG. 5.—Western white pine age classes, 1924: A, 2-0 stock; B, 1-2 stock; C, 3-0 stock

That the 2-0 stock survived least well was not surprising, for its inferior development was apparent. (See Table 3 and fig. 5.) At first glance, the 3-0 stock seemed to be better developed than the 1-2 in most respects. However, the 3-0 stock had poorer balance because of its larger tops and slightly fewer roots than the 1-2. The fact that it had 15 per cent more of total plant weight in the tops than the 1-2 seems an adequate explanation of its inferior survival.

The indicated superiority of 1-2 over 2-0 stock is in full agreement with the results attained 10 years earlier in the tests at Priest River and Wallace, Idaho. In the experiment at Haugan the survival of the 3-0 stock was intermediate between those of 1-2 and 2-0, corresponding to the 2-1 stock in the experiment at Wallace. This suggests that much the same results may be expected from 2-1 and 3-0 stock, although no comparative tests have as yet been made. From the tests actually made, the western white pine age classes rank in this order in ability to survive field planting: 2-2, 1-2, 2-1, or 3-0, 2-0, and 1-1. But when cost of survival is considered, 2-0 stock leads on the less severe planting sites. Of the other possible age classes, 1-year-old trees are too small, and 4-year-old seedlings or 5-year-old transplants are too large for field planting by present methods.

#### TESTS OF SIZE CLASSES

Strictly speaking, the best size of trees for planting can not be considered independently of the method of planting. Large trees can not be removed from the nursery without the loss of many roots, nor can they be properly planted except by more expensive methods than those ordinarily employed. Also there is a point beyond which the reduction in size of planting stock is not economical. In most places the trees in forest plantations have to fight drought, fungus diseases, rodents, insects, or any of a variety of enemies. Except in very favorable localities, destructive influences limit the reduction in size of planting material and preclude the possibility of success from the sowing of seeds directly in the field (7). The study of the best sizes of trees for planting has thus been restricted within definite limits.

TABLE 5.—Average tree measurements at the time of planting 1-2 western yellow pines, at Priest River, Idaho

Size class	Length of root	Length of top	Diameter of stem
	<i>Inches</i>	<i>Inches</i>	<i>Milli- meters</i>
Large.....	8.2	3.8	3.3
Medium.....	9.0	3.3	2.4
Small.....	7.8	2.7	2.0

#### WESTERN YELLOW PINE

Plantations on flat ground at the Priest River station in the fall of 1913 and spring of 1914 were made with 1-2 western yellow pines from the Savenac Nursery.<sup>6</sup> The trees were sorted into three size

<sup>6</sup> All of the experiments at Priest River station were planned and executed by J. A. Larsen. The work was entirely independent and separate from that at Wallace, Idaho, and Haugan, Mont., but is discussed here as it is within the region and essentially a part of the subject with which this article deals.

classes, the large and well-developed plants being placed in one group, the noticeably small and underdeveloped in another group, and the remaining medium-sized trees in a third group. This grading resulted in 524 large, 978 medium, and 824 small trees. An idea of the relative size of these trees may be obtained from Table 5, which gives the average measurements of 50 mechanically selected representatives of each size class. Planting was done by the so-called prepared-side-hole method. This differs from the slit method principally in that the soil is loosened by a few strokes of the mattock before the hole is made and the tree inserted. The results of this work are shown in Table 6.

TABLE 6.—*Survival and height growth of 1-2 western yellow pines according to size classes*<sup>a</sup>

Size of trees and season of planting	Survival in different years						Height in different years					
	1914	1915	1916	1917	1919	1921	1914	1915	1916	1917	1919	1921
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
Planted in fall:												
Small.....	52	44	42	41	41	41	4	5	9	12	20	31
Medium.....	50	44	42	42	42	42	2	6	9	13	18	32
Large.....	65	60	57	57	57	54	5	7	11	15	24	37
Planted in spring:												
Small.....	70	51	49	44	44	44	3	4	8	10	18	34
Medium.....	74	60	58	56	52	52	4	5	9	12	22	33
Large.....	89	79	79	76	73	73	5	6	12	16	26	38

<sup>a</sup> Plantations made in the fall of 1913 and spring of 1914 on flat land at Priest River, Idaho.

The large trees survived best in both plantations, and following spring planting the medium-sized trees survived better than the small trees. Spring planting resulted in higher survival than fall planting. In both plantings the small and medium-sized trees grew at about equal rates and were surpassed by the large grade. The thrifty appearance of this plantation of large trees after nine years' growth is shown in Figure 6, A. Although this experiment clearly indicates that size of trees greatly influences results, it gives no definite basis for judging the portion of stock that should be rejected before planting in the field.

Greater refinements in grading into size classes were attempted in the spring of 1915, with the view of making it possible to give specific recommendations regarding the proper degree of culling necessary to exclude weak plants. A lot of 4,300 trees were classified into the eight grades illustrated in Figure 7, A, and were planted in the spring of 1915 on a flat and on a southwest aspect.

The relative survival of the trees is indicated in Figure 8. The results on the flat show some tendency toward a reversal of those on the slope, but the order is confused. The uniformly higher survival on the flat is taken as an indication of better soil moisture conditions there than on the slope. The more severe conditions on the slope bring out clearly the inferiority of the lowest grade or smallest 5 per cent of the stock. The smallest 20 per cent of the whole (or lots numbered 1, 2, 3, and 4) remained constantly lower in survival than the 80 per cent of larger plants. Average growth measurements showed that the growth rate increased steadily with the

size of trees planted until, in 1921, the trees from the largest grade were 1 foot taller than those of the smallest grade. The results indicate that although no trees of the grades tested need be rejected

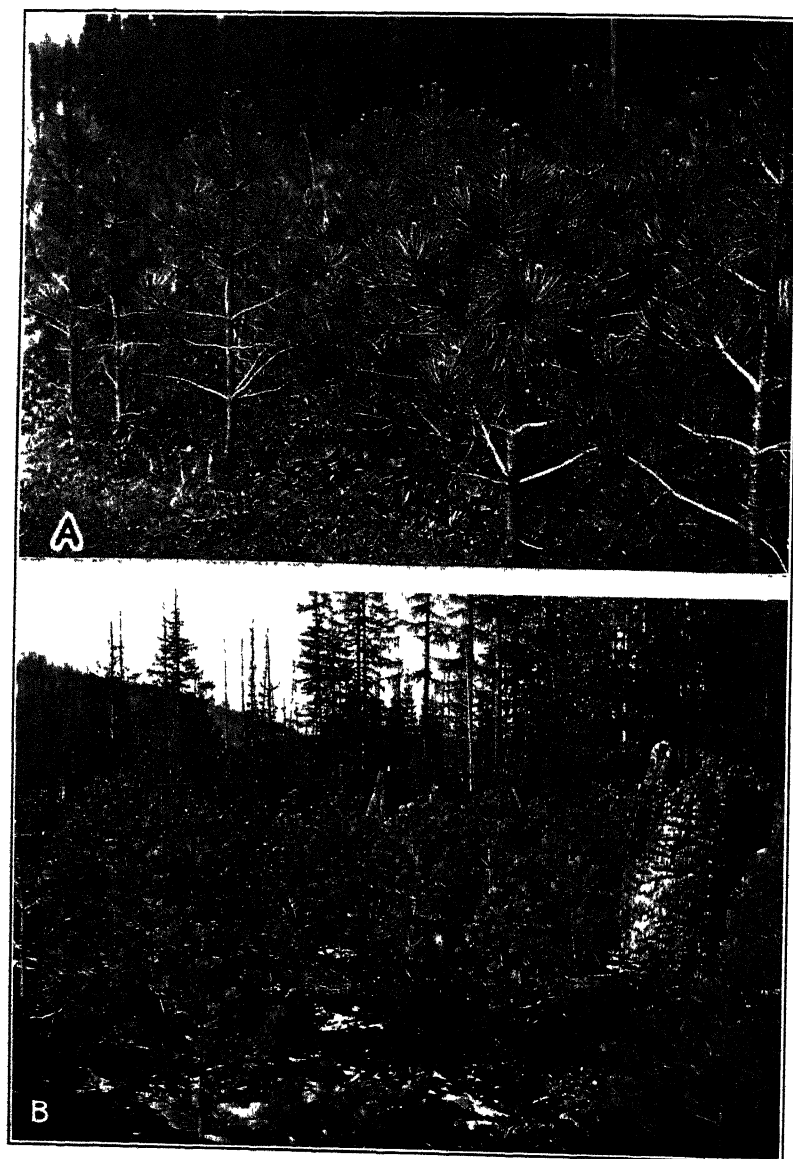


FIG. 6.—Highly satisfactory plantations of 1-2 stock: A, 9-year-old plantation of large-grade western yellow pine stock, trees about 3.5 feet tall, 1922; B, 8-year-old plantation of large-grade western white pine, trees about 2 feet tall, 1922

for planting on favorable sites, about one-fifth of the trees should be culled out before planting the drier slopes.

In May, 1923, about 750 1-2 western yellow pines were lifted at the Savenac Nursery for a test of size classes. After rejecting 5

per cent of these because of poor development or injury, the lot was graded into three classes, large, medium, and small, each consisting of one-third of the total number. Roots of the large plants were pruned to a length of 7 inches, the medium size to 6.5 inches, and

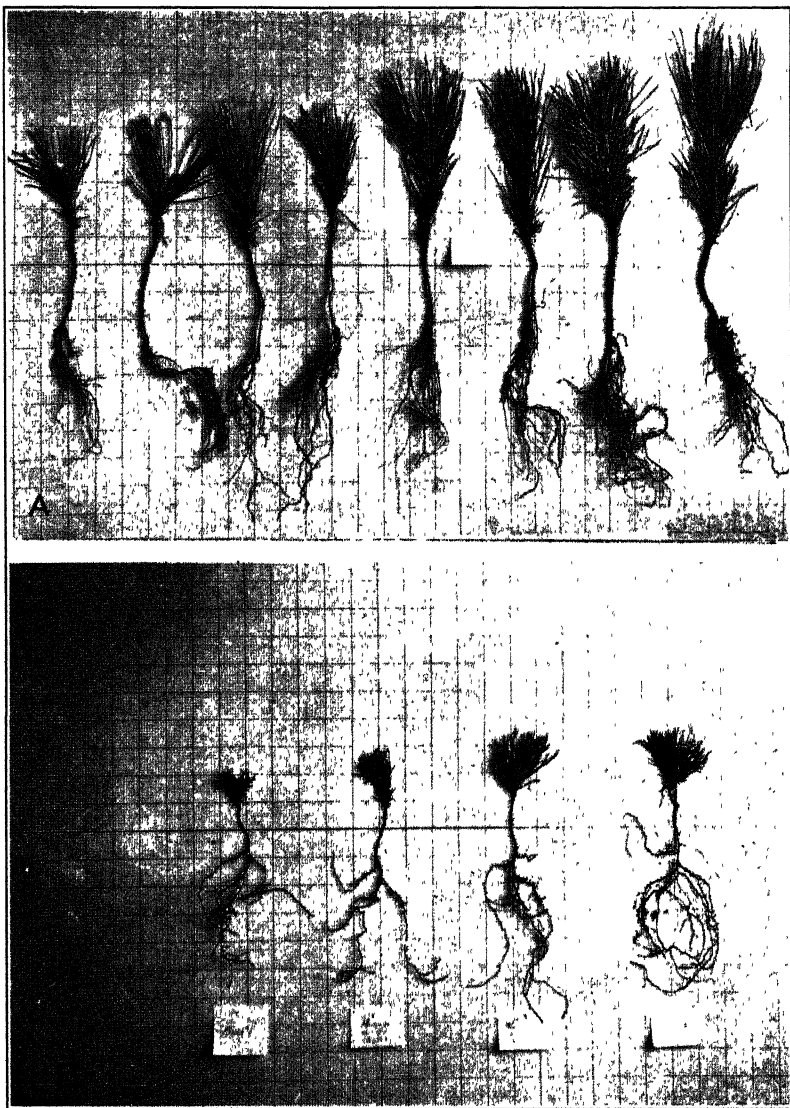


FIG. 7.—Graded sizes of 1-2 planting stock: A, Eight grades of western yellow pine planted in the spring of 1915; B, four grades of western white pine planted in the fall of 1914 and the spring of 1914

the small to 6 inches, in order to maintain as nearly as possible the same balance in the different lots. The figures in Table 7, giving percentage of total plant weight contained in the tops, show this effort to have been successful. The table also shows in detail the difference in size of the plants. Early in 1923 the small grade showed

the largest number of unthrifty plants, the large grade the fewest, and the medium grade an intermediate number, but survival was uniformly high until drought occurred in September. On the seventh day of the month the grades ranked as follows in survival: Large, 90 per cent; medium, 80 per cent; and small, 62 per cent. Each size had the benefit of roots one-half inch longer than those of the next smaller size. Undoubtedly this influenced the results obtained, but as the deeper layers of soil within the root zone did not become dry until September, it does not seem likely that root length alone was responsible for any more of the differences in survival than were the other characteristics shown in Table 7. Mortality during the

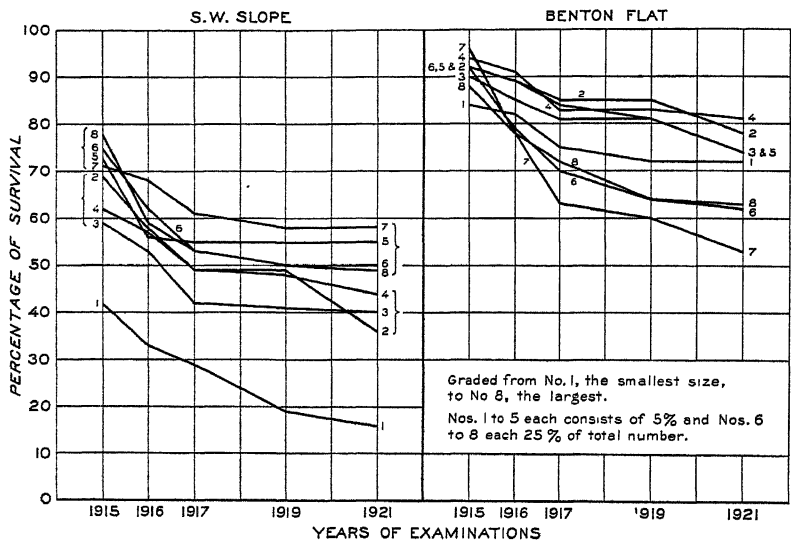


FIG. 8.—Size classes, series F, graded 1-2 western yellow pine stock, basis 4,300 plants; plantation of spring of 1916, Priest River Experiment Station, Idaho

second season was also less for the larger sizes. In October, 1924, survival was as follows: Large, 82 per cent; medium, 68 per cent; and small, 45 per cent.

TABLE 7.—Anatomical characteristics of the root-pruned 1-2 western yellow pine size classes, as determined by average measurement <sup>a</sup>

Size class	Stem length	Length of needles	Diameter at ground line	Lateral rootlets		Oven-dry weight	Weight in top	Weight in root
				0.5-2 inches	Over 2 inches			
	Inches	Inches	Milli-meters	Number	Number	Grams	Per cent	Per cent
Large.....	4.1	3.5	3.6	27.6	15.1	2.6	72	28
Medium.....	3.4	3.3	3.0	14.8	9.1	1.6	72	28
Small.....	2.6	2.6	2.2	8.0	4.9	0.8	73	27

<sup>a</sup> Planted in May, 1923, near Haugan, Mont. Averages based on measurements of 60 trees of each size class.



These results were corroborated in 1924 by the results of a plantation containing over 1,175 western yellow pines in each of the size classes—large, medium, and small. In April, the trees were planted on a steep, gravelly south slope where conditions of plant growth were obviously severe. Five examinations during the season showed that survival was constantly highest for the large, intermediate for the medium, and lowest for the small stock, but the differences were not large and by the end of the season less than 15 per cent of the plants were alive in each grade. The combined effect of a severe site and a dry year was the cause of the failure to obtain satisfactory survival. The foregoing results agree with those obtained by Show (5) in California, who found that graded sizes of 1-2 western yellow

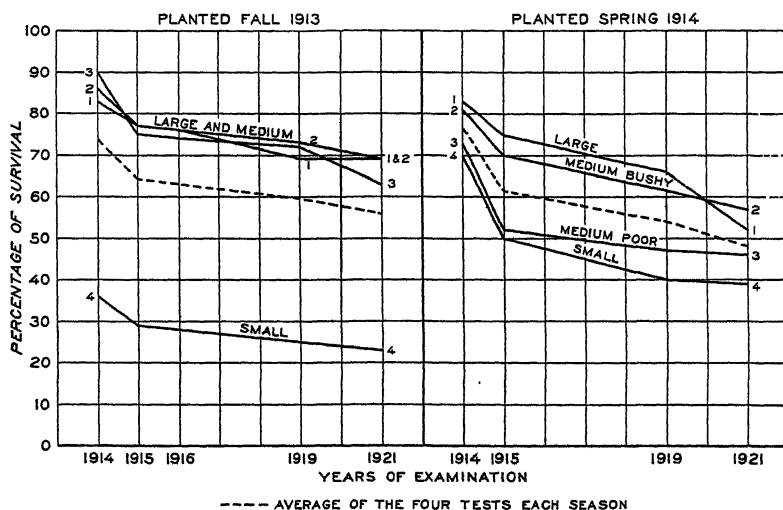


FIG. 9.—Size classes, series A, graded 1-2 western white pine stock, about 400 plants in each test; plantation on Benton Flat, Priest River Experiment Station, Idaho

pine survived as follows in 1916: Large, 50 per cent; medium, 38 per cent; and small, 23 per cent.

#### WESTERN WHITE PINE

Experiments similar to those with western yellow pine were made with the western white pine at the Priest River station in the fall of 1913 and spring of 1914, using 1-2 stock grown at Savenac Nursery. This time the trees were sorted into four grades: (1) Large stock with large, well-developed roots; (2) medium-sized trees with well-developed roots; (3) medium-sized trees with poor and stringy roots; and (4) small plants. About 40 plants were mechanically selected from each grade for the measurements given in Table 8; representative trees are shown in Figure 7, B. The trends of survival in these plantations are shown in Figure 9.

A quantity of moisture greater than normal was available in the summers of 1914, 1915, and 1916, so that differences in drought hardness of the various classes do not stand out clearly in all cases. Of the lots planted in the fall the smallest size did very poorly, while the other grades were all much alike. In the fall plantation

survival was on the average higher than in the spring plantation. In the spring plantation the relative order of survival of the grades was constant for six years, showing the tendency toward higher survival of the larger plants. Measurements of total height in 1921 showed that the small class averaged about 4.5 inches shorter than the others, which were all about 22 inches high. (Fig. 6, B.)

TABLE 8.—Average tree measurements of 1-2 western white pines at the time of planting (fall, 1913, and spring, 1914) at Priest River, Idaho

Size class	Length of root	Length of top	Diameter of stem
	<i>Inches</i>	<i>Inches</i>	<i>Millimeters</i>
Large class.....	8.4	2.7	2.5
Medium with bushy roots.....	6.5	2.4	2.1
Medium with stringy roots.....	6.9	2.2	1.9
Small class.....	6.3	2.0	1.5

### PRUNING TOPS TO MAINTAIN BALANCE

The term "balance" as used in speaking of forest-planting material means the relation between the capacity of the root system to absorb moisture and the capacity of the crown or top to transpire moisture, which in turn is a measure of the water use of plants. It is true that other things besides balance influence the economical use of water by plants. Bates (1) has shown how the water requirements of trees vary with different species. Numerous external factors influence the water-supplying power of the soil and the evaporating power of the air, thus controlling the flow of water through a plant. But balance, as an attribute of the plant itself, is to a certain extent capable of modification in the nursery.

It is the aim of forest nursery practice to produce plants with tops as small as possible in comparison with the usable portion of the root system. As a rule transplants have much better balance than seedlings that have never had their roots pruned during their period of development, and the usual higher survival of transplants is largely ascribed to their smaller top-root ratio. If differences in survival can correctly be attributed to corresponding differences in balance, as was done with the test of western yellow pine age classes planted in the spring of 1923, it is essential that balance be measured by as precise a method as is practicable. In transpiration tests made in 1917, Bates (1) computed the area of leaf surface from needle dimensions and volume displacements, but he recognized the error due to the inclusion of stem volume. Similarly, the weight of wood in stems and roots is a source of error in judging balance from measurements of weight alone. Yet simple comparisons of oven-dry weights are easily made, and the unavoidable error should not destroy the value of the method unless there be great variation in size of the trees compared. Because the method of weight comparison can be applied to representative lots of 100 or more nursery trees at one time, and is easy to use, it is preferred to the "leaf exposure" method.

The balance of trees taken from forest nurseries is almost invariably inferior to that of naturally grown trees of the same age. This is because the root systems penetrate the nursery soil so deeply that

they are seldom removed intact, and also because the especially long roots that did not happen to break off in the soil are pruned off previous to planting in order to prevent them from being doubled up in the planting holes. With hardwoods top pruning is commonly practiced in order to restore the balance that is lost in root pruning. In horticultural practice, because symmetrical tops are desired, the tops of conifers are not pruned, but symmetry is nonessential to forest trees less than 4 years old. Light and severe pruning of needles with both white and yellow pine has been tested in field plantations near Hagan, Mont. Although the results were negative, a few words about the tests may interest those who may have contemplated similar trials.

In preparing 1-2 western yellow pine for planting in April, 1923, certain lots of trees were top pruned. By cutting horizontally with shears, the uppermost portions of needles were removed, leaving only the part of the crown that could not be removed in one cut without injury to the terminal bud. This resulted in plants with about 10 per cent less of their total dry weight in the tops, an improvement in balance that appeared sufficient to increase field survival. No excessive bleeding of sap from the cut ends was observed; the needles promptly sealed their wounds, and the dying of needle tissue progressed only about one-sixteenth inch from the cut ends. Nevertheless, the vitality of the plants must have been reduced in some way by the pruning, because survival was lowered in spite of the improved balance of the trees. Too severe reduction of the organs of photosynthesis is a plausible explanation of the injury. This idea was strengthened by the field observation that the plants that died were principally the smallest ones, which lost the largest proportion of their crowns, and the observation that numerous very thrifty plants were all among the large trees with very slight or no top pruning.

Probably the reduction of the plants' equipment for assimilating food was especially injurious because it was made early in spring, the season of naturally rapid growth. Later pruning might help the plant through the critical period of summer drought without this early interference with vital functions, but of course it could not be considered as a separate operation in practice. The question arose as to whether pruning of tops might be beneficial in connection with late planting. Heavy and light top pruning was tried in both early and late plantations. The lightly pruned trees in the late plantation did best of all, but were not sufficiently superior to the unpruned trees to form evidence in favor of the theory under test. In general, pruning lowered survival.

#### PROPER LENGTH OF ROOT SYSTEM

The benefits from deep setting of roots on exposed sites have been mentioned by Toumey (6). From 1921 to 1924 the following theory along the same line was tested by various experiments: The heavy drought losses in the forest plantations the first season following plantings are largely due to the downward desiccation of the soil during the dry season, the soil often becoming devoid of available moisture beneath the lowermost level reached by the roots. Seedlings arising from natural reproduction immediately extend their roots downward in an attempt to keep ahead of the advancing dry

soil, but planted trees fail to do this with sufficient promptness. Owing to the shock of planting, the downward penetration of roots during the first season is negligible.

A preliminary test was made with 2-0 western white pine in the spring of 1921. Six hundred seedlings were root-pruned at points 8 inches from the ground line and planted on a northwest slope together with 400 seedlings with unpruned roots. Holes were made sufficiently deep to accommodate the roots of each plant without any doubling back. This gave the unpruned lot the advantage of about  $1\frac{1}{2}$  inches greater depth of roots. By the end of September, 43 per cent of the unpruned trees and 59 per cent of the pruned trees were dead. Nearly all of the deaths occurred during July and August and were attributed to drought.

With trees planted in the spring of 1923 more thorough tests were made of the influence of root length on survival. Of 1-2 western yellow pines lifted from the nursery in the usual manner, 5 per cent were rejected because of root injury and 2 per cent because of poor development. Many but not all of the trees retained roots 10 inches long, and variation in balance was apparent. Accordingly, as trees were pruned individually at points 4, 6, 8, and 10 inches below the ground line, they were also graded sufficiently so that the larger trees were pruned to the longer and smaller trees to the shorter lengths. This prevented the larger trees from becoming extremely top-heavy. In general, the procedure tended to equalize the balance of the four lots.

The trees were planted in April, 1923, near Haugan, Mont., on a southwest slope having soil of medium quality. During that season this plantation was examined six times at monthly intervals, thus affording opportunity to observe evidences of external causes of injury while they were still fresh. All such causes, other than drought, which appeared to contribute to upthriftiness or death of individual trees were noted and the record of such trees was excluded from further considerations. The number of trees so excluded was not more than 4 per cent of any lot and did not seriously reduce the basis for conclusions.

A study of soil moisture revealed some points of interest. At monthly intervals 10 soil samples were taken to represent the soil at each of two depths, 4 and 10 inches, in different parts of the plantation. As determined in another experiment, the wilting coefficient for western yellow pine is about 3.9 per cent of the weight of dry soil. Applying this figure to the results obtained from field samples permits their expression in terms of moisture available to western yellow pine. (See Table 9.) On July 3 the soil within reach of the trees appeared quite uniformly moist, whereas a month later the top layers had dried out until the soil at the 4-inch level contained only about one-third the amount of moisture available at the 10-inch level. The rains of August then added nearly enough moisture to the upper layers to offset further losses, but did not penetrate as deeply as 10 inches. However, the continued dampness of the soil about 10 inches from the surface for a month after the upper soil was dry had a perceptible effect on the trees.

TABLE 9.—Soil-moisture determinations at 4 and 10 inch depths

Date	Moisture at 4-inch depth		Moisture at 10-inch depth	
	Total	Available	Total	Available
July 3.....	<i>Per cent</i> 18.9	<i>Per cent</i> 15.0	<i>Per cent</i> 18.6	<i>Per cent</i> 14.7
August 3.....	7.4	3.5	15.5	11.6
September 3.....	6.6	2.7	6.2	2.3
October 3.....	6.3	2.4	5.9	2.0

Soil-moisture losses were greater than gains during the summer, but the precipitation for the season was above normal in amount each month until September, and until then the survival of all lots of trees remained high. A 20-day rainless period accompanied by high

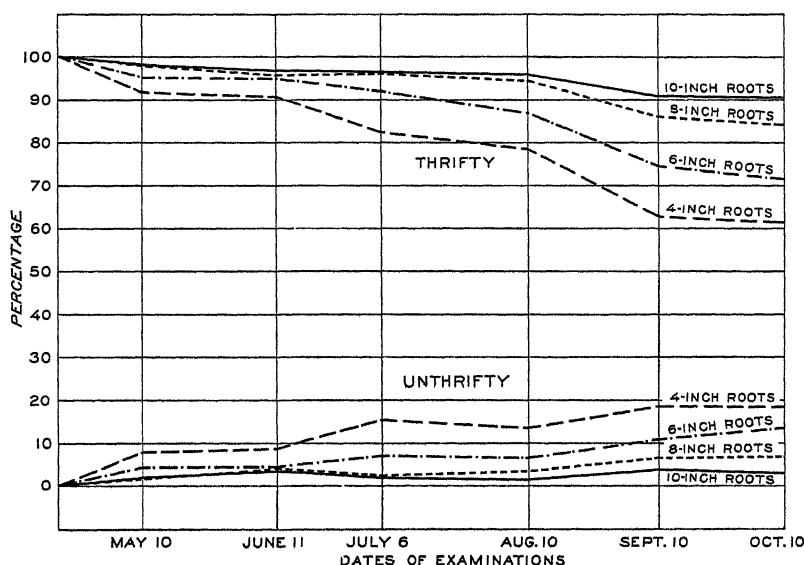


FIG. 10.—Root-length test: Relative thrift during 1923 in the 1-2 western yellow pine plantation of the spring of 1923; stock graded to preserve balance; roots measured and pruned individually; plants affected by causes other than dryness are not considered; southwest aspect

temperatures started on August 27, causing an acceleration of the death rate in plantations. Drought hardness increased with the length of roots. At the end of the season figures of the percentage of survival for the different root-length classes were as follows: 4 inch, 80 per cent; 6-inch, 85 per cent; 8-inch, 91 per cent; and 10-inch, 94 per cent. Although these percentages, owing to the moist season, are all unusually high, they serve the purpose of the experiment in showing clearly and consistently the influence of length of roots. From observations made at intervals figures were compiled showing the relative number of thrifty and unthrifty trees in percentage of the total number planted. These figures, plotted in Figure 10, show clearly that throughout the season the trees with short roots were less thrifty than those with longer roots.

A year later the relative survival was the same as at the end of the first season, the differences amounting to 6 or 7 per cent for each 2-inch increase of root length. Measurements of current stem growth

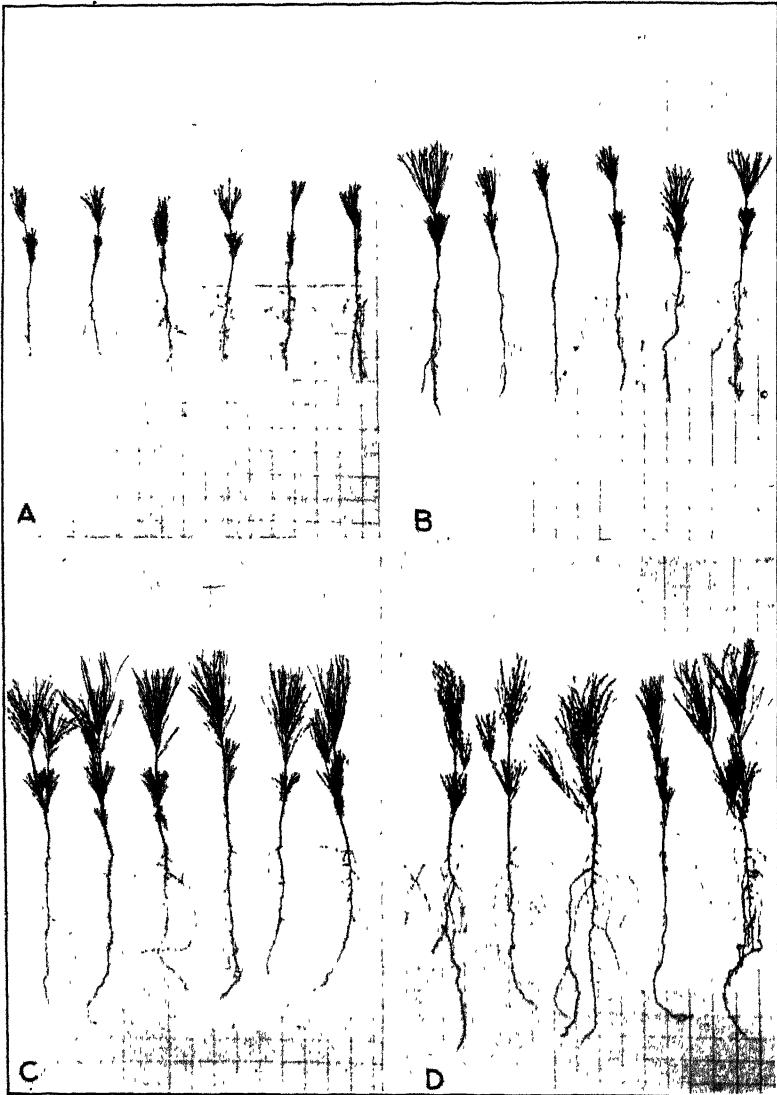


FIG. 11.—Western white pine root lengths. A, 4-inch class; B, 6-inch; C, 8-inch; and D, 10-inch

showed small but consistent increases in growth for the longer root lengths.

In 1923 a very similar experiment was made with 3-0 western white pines planted on a northwest slope. The trees are illustrated in Figure 11. The results were much like those attained with western yellow pine.

In weighing the results, the probable effect of the preliminary grading that tended to equalize the balance should be considered. Had the grading been omitted the long-rooted trees would have been less top-heavy and the short-rooted trees more so. Differences in survival of the various root-length classes might well have been still greater had it not been for the preliminary grading.

In sowings made in April, 1924, it was considered that in actual practice in pruning roots to certain lengths thorough grading will rarely precede the pruning of roots, and therefore no attention was paid to size and development of trees. More than 1,250 1-2 western yellow pines from each of three root-length classes, 6, 8, and 10 inches, were planted on a gravelly south slope presenting severe conditions for plant life. By the end of June the soil had become quite dry and but 63 per cent of those with 6-inch roots, 76 per cent of those with 8-inch roots, and 83 per cent of those with 10-inch roots were alive. By the end of July, the driest month, survival was as follows: Those with 6-inch roots, 14 per cent; 8-inch roots, 27 per cent; and 10-inch roots, 36 per cent.

A post-mortem examination of numerous trees indicated that the difficulty of planting properly in a loose gravelly soil and on a brushy site had resulted in a loss of the desired depth of root penetration at the time the trees were set out. Owing to slight root curvature, doubling back, inclination, or depth of setting, the average penetration was about 0.5 inch less than was intended, the different lots being affected differently. The 6-inch roots had lost about one-quarter inch, and for each additional 2 inches of root length, the loss of depth increased about a quarter inch. In other words, the trees with 6-inch roots actually penetrated 5.75 inches, the trees with 8-inch roots, 7.5 inches, and those with 10-inch roots, 9.25 inches, making the interval 1.75, rather than 2 inches. This condition did not affect the applicability of the results, because a better quality of planting could not be expected on such sites. However, it served to show that low survival on severe sites may be due not only to direct action of the site factors but also to the indirect action of the site in decreasing the quality of planting. A similar observation was made in California by Show (5), who found that quality of planting decreased with the increase in density of brush cover.

The four experiments described above indicate consistently that, within the limits tested, increases in the length of roots planted result in corresponding increases in survival.

#### SUMMARY

The results of the experiments with western yellow and western white pine showed that, other factors being equal, large stock survived better than small stock, that transplants are usually preferable to seedlings, that stock with roots 8 inches long or longer succeed better than stock with shorter roots, and that a low top-root ratio indicates better planting stock than a high ratio.

A higher proportional survival was found in the older age classes of both species. In one planting upon northwest, west, and southwest slopes, 1-2 stock of western yellow pine grew most rapidly from the start and also appeared the most economical from the standpoint

of the cost per unit of surviving trees. In another test, comparing survival of two age classes, 2-1 stock did better than 1-2, but 1-2 still remained the more economical. In one western white-pine planting 1-2 stock ranked superior to 2-0. In another planting of white pine in which 2-2 stock was used, this stock had the highest survival, with 1-2, 2-1, 2-0, and 1-1, in descending order. Upon the basis of cost per unit of survival 2-0 white-pine stock appeared most economical on moderate sites, but on the more severe sites 1-2 stock, or possibly 2-2, proved the least expensive.

In several experiments with size classes large stock of western yellow pine survived better than small stock on severe sites. Medium-sized stock appeared best on favorable sites. When western white-pine stock graded into four size classes was planted, only the smallest class resulted in poor survival.

When 1-2 western yellow-pine stock was root pruned to furnish root lengths of 10, 8, 6, and 4 inches, highest survival attended the stock with 10-inch roots. The survival declined quite uniformly with the decrease in root lengths regardless of whether the stock has been sorted into size classes so that the root pruning was in proportion to the total size of the stock or not. Roots 8 inches or longer seemed best.

In general the season of planting appears to have slight effect upon survival.

#### LITERATURE CITED

- (1) BATES, C. G.  
1923. PHYSIOLOGICAL REQUIREMENTS OF ROCKY MOUNTAIN TREES. *Jour. Agr. Research* 24: 97-164, illus.
- (2) HENRY, A. J., KINCER, J. B., FRANKENFIELD, H. C., GREGG, W. R., SMITH, B. B., and MUNNS, E. N.  
1925. WEATHER AND AGRICULTURE. U. S. Dept. Agr. Yearbook 1924: 457-557, illus.
- (3) KORSTIAN, C. F., and BAKER, F. S.  
1925. FOREST PLANTING IN THE INTERMOUNTAIN REGION. U. S. Dept. Agr. Bul. 1264, 57 p., illus.
- (4) LARSEN, J. A., and DELAVAN, C. C.  
1922. CLIMATE AND FOREST FIRES IN MONTANA AND NORTHERN IDAHO, 1909 TO 1919. U. S. Mo. Weather Rev. 50: 55-68, illus.
- (5) SHOW, S. B.  
1924. SOME RESULTS OF EXPERIMENTAL FOREST PLANTING IN NORTHERN CALIFORNIA. *Ecology* 5: 83-94.
- (6) TOUMEY, J. W.  
1916. SEEDING AND PLANTING; A MANUAL FOR THE GUIDANCE OF FORESTRY STUDENTS, FORESTERS, NURSERYMEN, FOREST OWNERS, AND FARMERS. 455 p., illus. New York and London.
- (7) WAHLENBERG, W. G.  
1925. REFORESTATION BY SEED SOWING IN THE NORTHERN ROCKY MOUNTAINS. *Jour. Agr. Research* 30: 637-641, illus.
- (8) ———  
1926. AGE CLASSES OF WESTERN WHITE PINE PLANTING STOCK IN RELATION TO ASPECT OF PLANTING SITE IN NORTHERN IDAHO. *Jour. Agr. Research* 33: 611-619, illus.



# TWO NEW ALEYRODID (CITRUS) PESTS FROM INDIA AND THE SOUTH PACIFIC<sup>1</sup>

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This paper records two white-fly pests that are apparently very serious enemies of citrus in their respective countries. Great care should be taken to see that they are never introduced into other citrus-growing regions of the world, where they would undoubtedly prove to be citrus enemies of first rank.

While the following species of white-fly undoubtedly falls into Cockerell's genus *Dialeurodes*, a new subgenus must be erected for its inclusion. It differs greatly from the other members of the genus in its extremely elongate shape.



FIG. 1.—Citrus leaf infested with *Dialeurodes (Dialeurolonga) elongata* and *Aleuromigda* sp. Greatly enlarged

## ***Dialeurolonga*, new subgenus.**

Pupa case very elongate, more or less asymmetrical, yellowish, shallow corrugations or striations on the submarginal area with a row of papillalike pores present; dorsal disk without pores and with no development of rhachis; thoracic tracheal folds distinct, ending on margin in a pore; vasiform orifice rather elongate, the caudal margin toothed.

Type of subgenus.—*Dialeurodes (Dialeurolonga) elongata* Dozier

## ***Dialeurodes (Dialeurolonga) elongata*, new species.**

Easily distinguished from all other *Dialeurodes* by its elongate shape. (Fig. 1.) At a superficial glance it reminds one of *Bemisia giffardi*.

<sup>1</sup> Received for publication Apr. 7, 1928, issued August, 1928.

Pupa case (figs. 2 and 3) yellowish white, lying closely adpressed to the leaf surface; scattered indiscriminately on lower surface and greatly resembling a young Lecanium scale; without any kind of wax secretion.

Length 1.46 mm., greatest width 0.670 mm. Shape very elongate-elliptical but distinctly unsymmetrical in outline. Thoracic and caudal tracheal folds prominent, terminating in distinct pores. The entire surface apparently granulated in balsam mounts; the submarginal area set off by corrugations or striations, more whitish or transparent in color; a row of inconspicuous papillalike pores follows around the submarginal border; vasiform orifice rather elongate, the caudal margin toothed; the operculum similar in outline.

In balsam slide-mounted specimens, the color of the pupa case is a transparent yellowish white with two irregular patches of orange pigment on disk on each side of the abdominal region, and this orange coloring is very characteristic.

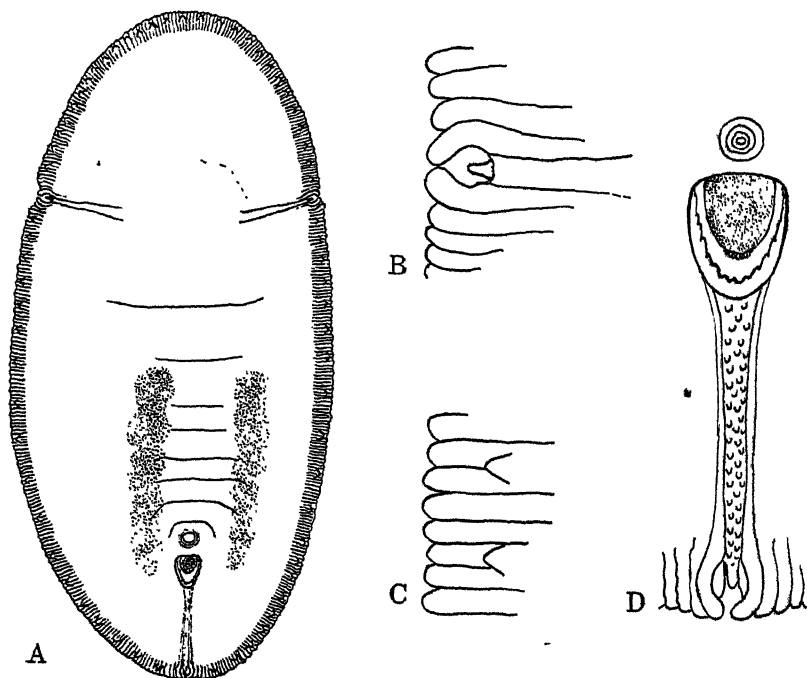


FIG. 2.—*Dialeurodes (Dialeurolonga) elongata*: A, Pupa case; B, thoracic tracheal pore; C, margin of pupa case; D, vasiform orifice and caudal pore

Described from abundant pupa-case material, slide mounted in Canada balsam and on citrus leaves, collected by M. Afzal Husain at Lyallpur, Punjab, India, March 29, 1926, No. 308.

Cotype slide-mounted material of the above-described new species is deposited in the collections of the United States National Museum, the British Museum, the California Citrus Experiment Station, the Florida State Plant Board, and the private collections of the author and of the Japanese authority on this family, S. I. Kuwana.

#### ALEUROPLATUS (ORCHAMUS) SAMOANUS LAING

This species on account of the vasiform orifice being armed with teeth falls at once into the subgenus *Orchamus*, and is closely allied to *Aleuroplatus mammaeferus* Q. & B. It is less elliptical than that

species, the marginal pores are different, the thoracic comb is more depressed, and the teeth are distinctly longer.

Pupa case (fig. 4) 0.828 mm. in length; greatest width 0.584 mm. Shape oval, constricted across the cephalic portion at the thoracic folds. Dorsal sutures very indistinct. The margin with very indistinct shallow rounded teeth; supplied with a pair of extremely minute setae on anterior margin and another pair on posterior margin that might easily be overlooked. Just within the margin all around is a row of prominent pores that at first glance under the high power of the microscope give the impression of being very toothlike. A pair of non-prominent spines or setae present on disk of dorsum. The marginal thoracic

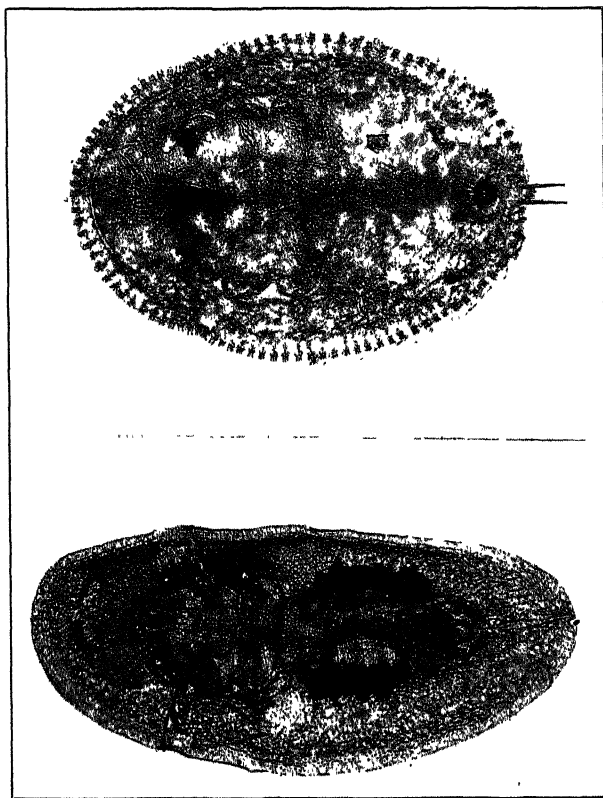


FIG. 3.—Photomicrographs of the pupa cases of *Dialeurodes elongata* and *Aleuroplatus samoanus*

comb distinctly depressed, very prominent, formed by long, narrow teeth, about 11 in number; the caudal fold is armed with a similar prominent comb composed of 9 teeth, and on each side of this comb is a long, distinct marginal seta. Vasi-form orifice broadly rounded, the lateral caudal margins armed with distinct teeth or folds. Color of case a transparent dirty or yellowish white.

On the leaf the pupa case appears slightly elevated, surrounded with a very broad, flattened, yellowish white waxy secretion which varies in width and irregularity of outline.

Adults unknown.

The above description was made from abundant pupa-case material on leaves of citron (*Citrus medica*), collected by the late F. L. Washburn in his expedition to the Marquesas Islands in the South Pacific in 1926. This material is preserved in two vials of

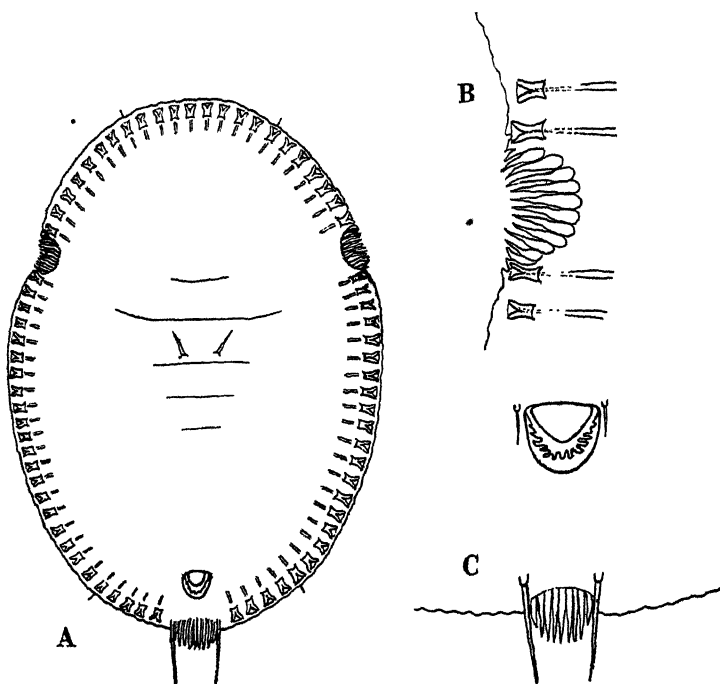


FIG. 4.—*Aleuroplatus (Orchamus) samoanus*: A, Pupa case; B, thoracic tracheal comb and wax pores; C, vasiiform orifice and caudal comb

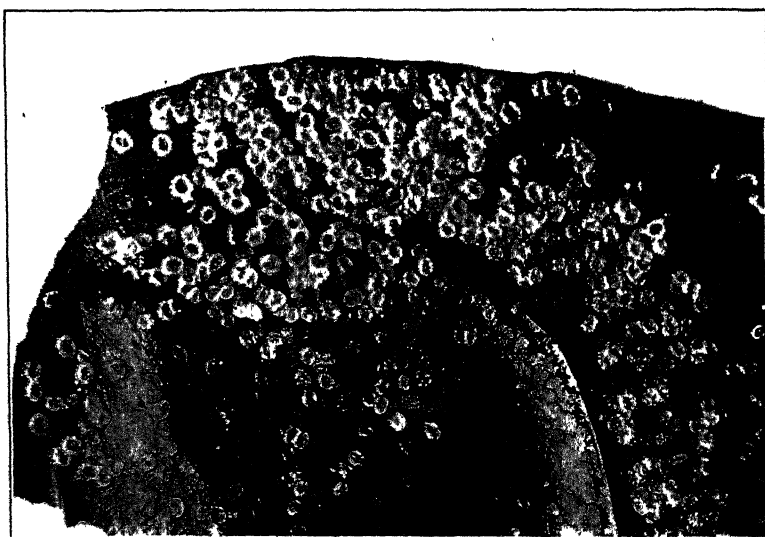


FIG. 5.—Citron leaf heavily infested with the white fly, *Aleuroplatus samoanus* L. Enlarged. Photograph made from formalin-preserved material

formalin, the cork of one of which is marked "H" and the other "T 8 citron." Clarence E. Mickel, who sent the writer this material for study, has been able to locate some notes left by Washburn that undoubtedly refer to this material. "I left San Francisco June, 1926, and arrived at Tahiti on June 26. I employed my time collecting in the vicinity of Ventura, 13 kilometers from Papeete, avoiding, as far as possible, duplication of the collection made on this island two years previously." He listed a few aleurodids among the material taken in this vicinity. "Hikueru was our next stop. Here I collected for eight days. The insect fauna on Hikueru was found to be practically identical to that at the atolls previously visited." While no mention is made of aleyrodids being taken on Hikueru, the "H" on the vial of material collected on the first trip in 1923 most probably refers to this island as Washburn states at the end of these notes that "H" equals Hikueru, collected in August.

The infested leaves in both of the vials are undoubtedly *Citrus* sp. and are extremely heavily infested. *Aleuroplatus samoanus* was described (Insects of Samoa, Pt. II, British Museum, 1927) from thickly coated leaves of cultivated Croton on Upolu Island, Apia, IV, 1925, and the material here discussed represents without much question that species.



# AN APPARATUS FOR OBTAINING MEASURED AREAS OF SPRAYED FOLIAGE FOR CHEMICAL ANALYSES<sup>1</sup>

By JOSEPH M. GINSBURG<sup>2</sup>

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## INTRODUCTION

In studying adhesion of insecticides and fungicides to foliage it is often necessary to determine the leaf area covered by the spray material before chemical analyses are made. For this purpose leaves are collected and the area of each one is determined separately. The measurements are made either from the fresh leaves directly or from blue prints, usually by the aid of a planimeter. Anyone who has had an opportunity to measure foliage by this method will admit that the work is rather tedious and time consuming, especially when a large number of leaves are to be measured. Besides, some of the spray material is bound to be shaken from the leaves during the process of measuring, particularly when the leaves are allowed to become dry before they are measured, thus providing a source for error in the subsequent chemical analyses. Again, experimental orchards are not always situated close to laboratory facilities and one or two days may often elapse from the time the leaves are cut until the measurements are taken, with the result that the exact area can not be obtained.

While engaged in an investigation of stickers and dilutents in arsenic-sulphur sprays and dusts, the writer tested out several methods for measuring the foliage surface in the field at the same time that the leaves are collected from the trees. After several efforts, the following apparatus was devised which gave satisfactory results.

## DESCRIPTION OF APPARATUS

The apparatus (fig. 1) consists of a steel base (*a*) from which is extended an arm (*b*) ending in a hollow sleeve (*c*). This entire part was cut out from one piece of solid steel. In the center of the base is a round opening (*d*),  $1\frac{3}{4}$  inches in diameter, into which steel dies (*i*) of various sizes can be fitted. Into the sleeve is inserted a plunger (*e*) around which winds a retarding spring (*f*). The lower end of the plunger is provided with an opening into which round cutters (*h*) of various diameters can be inserted and tightened with a flathead screw which passes through the plunger and grips the narrow end (*g*) of the cutter but does not touch the inner wall of the sleeve. This arrangement allows free movement of the plunger in the sleeve. The cutter is partially hollowed inside and tapers to a very fine, sharp edge so that when the cap of the plunger is pushed down it fits snugly into the die. This arrangement is absolutely necessary in order to obtain a clean cut of the leaf tissue and to cause the cut area to fall out promptly from the die without touching the upper surface of the cutter.

<sup>1</sup> Received for publication Apr. 26, 1928; issued August, 1928. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

<sup>2</sup> The author wishes to express his indebtedness to Dr. Thomas J. Headlee for helpful suggestions given during the construction of the apparatus.

The leaf is inserted between the die and the cutter and the cap of the plunger forced with the left hand, the arm of the apparatus being held in the right hand, until it hits the die. A round area of leaf tissue is thus cut out which automatically falls into a paper bag, or any other suitable container, attached to the base of the apparatus. During this operation the surface of the leaf is not touched by either the die or the cutter, except at the edges where the cut is made, and no loss of spray material occurs. As soon as the plunger is released the retarding spring causes it to move upward to its original position ready for another cutting. Each time the plunger moves upward the disk of the cutter raises a lever (*k*), extended from a small, three-figure counter (*j*) attached to the base of the apparatus. By this device every leaf-cut is automatically registered on the small counter

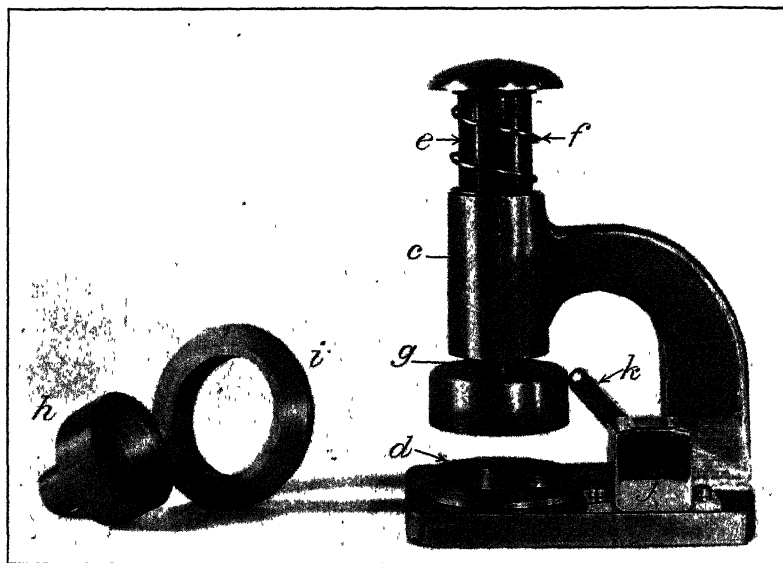


FIG 1.—Apparatus with automatic counter: *a*, Steel base; *b*, plunger arm; *c*, hollow sleeve; *d*, round opening to hold dies; *e*, plunger; *f*, retarding spring; *g*, narrow end of cutter; *h*, cutter; *i*, die; *j*, automatic counter; and *k*, lever connecting plunger with counter

and the total leaf surface collected can be calculated by multiplying the number of leaf areas cut (read from the counter) by the area of the cutter used.

As may be seen from the photograph of the apparatus, the cutter, and of course the die, can be readily removed from the apparatus and others of any desired size inserted. The cutters used in this apparatus were of two sizes. The larger size has a diameter of 1.5 inches and cuts a leaf area of 1.78 square inches, while the smaller one is only 1 inch in diameter and cuts a leaf area of 0.79 square inch. By the aid of this apparatus any desired total leaf surface can be obtained for chemical analysis by merely calculating the number of round leaf areas required to make up that surface.

#### CHEMICAL ANALYSES

The question came up as to whether or not the amount of chemicals recovered from the round areas fairly represents the amount on the entire leaves. To determine this point several samples of foliage



from two sprayed apple trees were collected and analyzed for arsenic and sulphur. The apple trees had been sprayed in the earlier part of the summer with spray mixtures containing 8 pounds of sulphur,  $1\frac{1}{2}$  pounds of lead arsenate, and various proportions of ferric oxide. The samples were collected during August and September. While cutting leaf samples with the apparatus, care was taken to obtain areas near the edges as well as from the central parts of leaves in order to secure representative samples.

The leaves were weighed, dried to constant weight at  $65^{\circ}\text{C}$ ., ground to a fine powder, and stored in tightly stoppered glass bottles for chemical analyses. The arsenic analysis was carried out by the modified Gutzeit method<sup>3</sup> while the sulphur was determined gravimetrically according to the Official Methods,<sup>4</sup> the fusion being made in an electrically heated muffle furnace. Averages of duplicate determinations, calculated in milligrams of  $\text{As}_2\text{O}_3$  and  $\text{SO}_3$  per gram of green leaves, are given in Table 1.

TABLE 1.—Amounts of  $\text{As}_2\text{O}_3$  and  $\text{SO}_3$  found in apple foliage when determinations were made on entire leaves and on round areas cut with the apparatus

Leaf sample No.	Date collected	Milligrams of $\text{As}_2\text{O}_3$ per gram of green foliage in—		Milligrams of $\text{SO}_3$ per gram of green foliage in—	
		Entire leaves	Round areas	Entire leaves	Round areas
30	Aug. 25, 1927..	0.140	0.149	2.80	2.50
31	Sept. 3, 1927..	.125	.129	3.10	3.40
32	Aug. 10, 1927..	.073	.070	Not determined	
33	Sept. 7, 1927..	.070	.071		

A comparison of the results secured from the two sets of samples does not reveal any appreciable differences between the quantities of arsenic or of sulphur found in the apple leaves. It appears therefore, that the apparatus herein described can be satisfactorily used in obtaining measured leaf areas for chemical analyses. By the aid of this apparatus the time and work required for planimeter measurements may be eliminated.

<sup>3</sup> SCOTT, W. W., STANDARD METHODS OF CHEMICAL ANALYSIS. p. 40. New York. 1917.

<sup>4</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C. 1920.



# THE TOUGHNESS OF COTTON BOLLS IN RELATION TO AGE AND NUTRIENT SUPPLY AS MEASURED BY PRESSURE TESTS<sup>1</sup>

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## INTRODUCTION

The fruiting responses of the cotton plant as influenced by several factors have been under investigation since 1924. Progressive changes in boll development have received attention, particularly the stage of maturation as determined by resistance to puncture.<sup>2</sup> This paper presents chiefly the results of the pressure tests on cotton bolls, with some data on boll size and wall thickness as associated with the stage of maturation. Data from pressure tests representing stages in the development of cotton fruits assume a somewhat different aspect from the results obtained with edible fruits, since with maturity the cotton boll dries and cracks, exposing a mass of lint. The boll period, or the number of days from the appearance of the bloom to the open boll, is known to be appreciably lengthened or shortened by seasonal conditions. These conditions undoubtedly affect both the physical and chemical processes involved in development. It was thought that the progress of some of these changes could be determined by a pressure test on bolls of known ages developed at different periods in the growth of the plant. Results were obtained during three seasons.

General observations have shown that cotton bolls are not seriously damaged by the cotton-boll weevil in their later stages of development, and it was thought that this resistance to attack with increasing age might be correlated with thickness and toughness of the boll wall.

Mechanical appliances for studying the maturity of fruits and vegetables have been used by a number of workers in the past few years, the idea being that alterations in the tissues with increasing maturity cause changes in the resistance to penetration. Lewis, Murneek, and Cate (8),<sup>3</sup> using a pressure tester on pears, state that the idea for the apparatus they describe was obtained from O. M. Morris, of the Washington Agricultural Experiment Station. According to the information available to the writer, Morris was the first to use a pressure tester on fruits. There have been other pressure tests (5, 15) on pears. A pressure test on apples has also been used by a number of workers (9, 10, 11, 14, 16).

One of the most accurate instruments for mechanical puncture tests is a modified Joly balance, which has been used by Hawkins and Harvey (6) on potatoes, by Rosenbaum and Sando (17) on

<sup>1</sup> Received for publication Apr. 9, 1928; issued August, 1928. The tests herein reported were conducted at the Pee Dee Experiment Station, Florence, S. C.

<sup>2</sup> Pressure tests on a total of 190 bolls of two varieties were made by C. A. Ludwig, at Clemson College, in 1923. The results and a picture of the apparatus were kindly furnished the writer before this work was begun; however, a different type of apparatus was developed for the present investigation.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 1024.

tomatoes, by Hawkins and Sando (7), and by Willaman, Pervier, and Triebold (19) on small fruits.

The puncture method to determine the toughness of the pericarp of sweet corn as related to maturity and other factors has been used by Rudnick and Bakke (18), by Culpepper and Magoon (1, 2), and by Magoon and Culpepper (12). All of these investigators report a progressive increase in the resistance of the pericarp to puncture with increasing maturity of the corn. The developing cotton boll, however, does not show a continuously increasing resistance to puncture as does the corn grain, for the boll may at times offer greater resistance to penetration at 21 or 27 days of age than at 41 days of age.

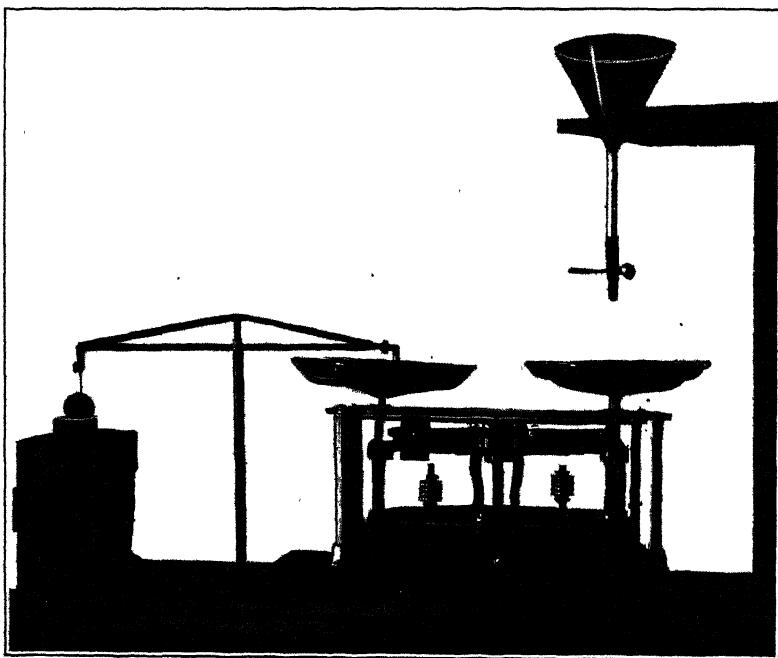


FIG. 1.—Apparatus used in 1924 for pressure tests on cotton bolls

#### METHOD OF PROCEDURE

The bolls were secured in 1924 from rows in the general cotton variety test. Tags bearing the date of appearance were attached to the white blossoms on plants of an outside row of each of eight varieties, and the bolls used for the pressure test were taken from these plants. In 1925 and 1926 treatment with a fertilizer relatively high in nitrogen was given to one of two adjoining half-acre plots, while a fertilizer relatively low in nitrogen was applied to the other plot. Two rows in each plot were selected for the tagging of blossoms and the subsequent removal of bolls.

Only a few bolls were taken from each plant, and these relatively late in the season, so that there was not the stimulation of vegetative growth which usually occurs when several bolls per plant are removed early in the season.

Samples of 10 bolls each were collected and puncture tests made immediately. The same sewing-machine needle, with the end so ground that a ball point penetrated the tissue, was used throughout the tests. The apparatus essentially as represented in Figure 1 was used in the tests of 1924. In actual operation it was found most convenient to place the boll in the pan, reverse the lever, and have the fixed end at the point where the needle and boll are represented. Sand from the funnel was run onto the pan of the torsion balance until the indicator made a quick movement, which showed the plunge of the needle into the tissue. The sand was then weighed. The apparatus illustrated in Figure 2 was used in 1926. The same pressure tester was used in 1925, but a slightly different arrangement did not permit quite the ease of operation as in 1926. The instrument was adapted from that illustrated by Culpepper and Magoon (*1*).

The wall of an immature cotton boll consists of three more or less distinct layers, a thin epidermis, a thicker spongy layer, and an inside fibrous-membranous layer. Pressure applied to the needle causes first a plunge as the epidermis is penetrated and then a second plunge as the inner layer is pierced. In cases where the plunge was not distinct, the test was repeated. Both pressures were recorded in 1924, but only the pressure necessary to penetrate the inner layer was recorded in 1925 and 1926. It appeared that the relative development of this layer was a better index of the stages leading to maturation, and it was certainly the only anatomical feature of the boll wall that would seem to offer sufficient resistance to boll weevil penetration to be of protective value. Each locule was punctured at two points on a line following the greatest circumference of the boll at right angles to the sutures. The side of the boll exposed to the sun is generally more highly colored than the unexposed side, and it was noted that the exposed side would frequently offer more resistance to puncture than the unexposed side. Since the bolls usually have from three to five locules, there were 6 to 10 tests per boll which made 62 to 98 tests for a 10-boll sample. The average of a 10-boll sample is used in all comparisons.

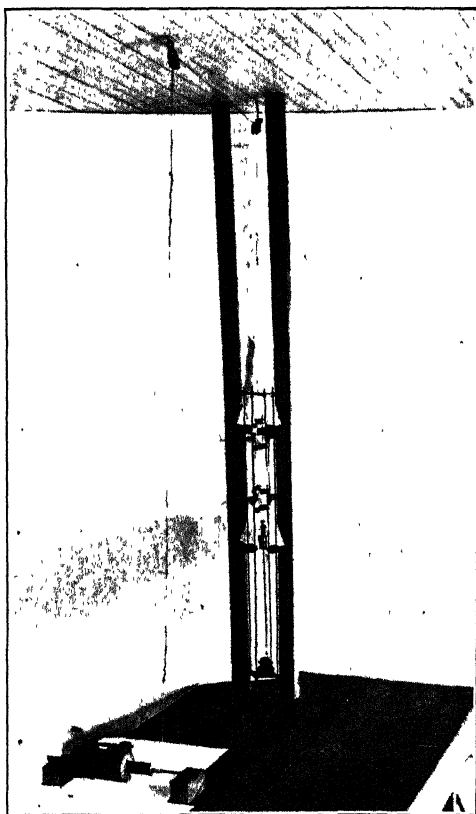


FIG. 2.—Apparatus used in 1925 and 1926 for pressure tests on cotton bolls

and 34 days of a single series. The same is true in general of the 34-day bolls of the two series. Thus it is seen that the age of the boll alone is not the chief factor in determining its toughness, or its resistance to puncture. The general trend of increase in toughness with age in the first series indicated that all varieties responded to the complex of seasonal conditions in much the same way. The variations in the second series seem to indicate either a distinct varietal response or an effect of conditions at or immediately preceding the time of the test. Bolls of the same age from all varieties were not tested on the same day in either the first series, with its more or less regularity in toughness of wall, or on the same day in the second series, with its irregularity in toughness of wall, which would indicate that the day of test is not the chief factor. Nevertheless the occurrence of such rather high figures as those for Carolina Webber and Wannamaker Cleveland on the last test of September 23, together with several such instances in 1925 and 1926, indicates that the seasonal conditions about the time of the test are sometimes of importance.

TABLE 1.—Results of an average puncture test upon 10 cotton bolls of 8 varieties at 27, 34, and 41 days of age, 1924

Series No.	Date of bloom	Date of test	Boll diameter	Age	Pressure <sup>a</sup>	Date of bloom	Date of test	Boll diameter	Age	Pressure <sup>a</sup>
Salsbury						Cleveland 12				
			<i>Cm.</i>	<i>Days</i>	<i>Grams</i>			<i>Cm.</i>	<i>Days</i>	<i>Grams</i>
1-----	July 16	Aug. 12	3.58	27	406	July 19	Aug. 15	3.63	27	<sup>b</sup> 439±5.1
	July 15	Aug. 18	3.56	34	407	July 16	Aug. 19	3.56	34	449±4.8
	July 25	Sept. 4	3.20	41	495	July 24	Sept. 3	3.56	41	495±5.1
2-----	July 26	Aug. 22	3.56	27	463	July 30	Aug. 26	3.63	27	479
	July 25	Aug. 28	3.56	34	468	July 29	Sept. 1	3.58	34	471
	Aug. 2	Sept. 12	3.38	41	450	Aug. 9	Sept. 19	3.45	41	442
Mexican Big Boll						Wannamaker Cleveland				
1-----	July 19	Aug. 15	3.34	27	427	July 18	Aug. 14	3.66	27	407
	July 17	Aug. 20	4.09	34	462	July 16	Aug. 19	3.53	34	451
	July 24	Sept. 3	3.56	41	466	July 23	Sept. 2	3.51	41	451
2-----	July 29	Aug. 25	3.78	27	445	July 31	Aug. 27	3.43	27	416
	July 26	Aug. 29	3.66	34	423	July 29	Sept. 1	3.43	34	456
	Aug. 3	Sept. 13	3.86	41	467	Aug. 13	Sept. 23	3.43	41	495
Lightning Express						Carolina Foster				
1-----	July 15	Aug. 11	3.43	27	409	July 16	Aug. 12	3.40	27	396
	July 16	Aug. 19	3.48	34	466	July 15	Aug. 18	3.40	34	411
	July 23	Sept. 2	3.38	41	496	July 26	Sept. 5	3.38	41	504
2-----	July 29	Aug. 25	3.30	27	449	July 30	Aug. 26	3.33	27	453
	July 27	Aug. 30	3.30	34	462	July 25	Aug. 28	3.25	34	450
	Aug. 10	Sept. 20	3.43	41	470	Aug. 2	Sept. 12	3.38	41	418
Carolina Webber						Dixie Triumph				
1-----	July 18	Aug. 14	3.51	27	<sup>b</sup> 431±4.9	July 19	Aug. 15	-----	27	<sup>b</sup> 433±4.6
	July 19	Aug. 22	3.38	34	445±4.3	July 18	Aug. 21	3.71	34	431±5.0
	July 25	Sept. 4	3.53	41	497±5.3	July 24	Sept. 3	3.38	41	470±5.1
2-----	July 31	Aug. 27	3.43	27	464	July 30	Aug. 26	3.56	27	462
	July 26	Aug. 29	3.48	34	473	July 29	Sept. 1	3.53	34	466
	Aug. 13	Sept. 23	3.40	41	566	Aug. 9	Sept. 19	3.39	41	443

<sup>a</sup> Each figure for pressure represents an average of 62 to 98 punctures of innermost boll wall.<sup>b</sup> Standard error.

Differences in rate of maturity may also be of importance in causing such high figures as those mentioned, since bolls will sometimes open when 40 to 45 days of age early in the season, whereas 60 to 70 days are required later.

Some varietal differences can be seen also in the eight varieties tested in 1924, though no one variety shows the least or greatest resistance to puncture in the bolls of all ages.

#### RESULTS OF PUNCTURE TESTS, 1925

The test in 1925 was confined to three varieties, Dixie Triumph, Cleveland 12, and Carolina Webber. The plants were grown under two conditions of fertility, namely, high nitrogen and low nitrogen, on adjoining one-half acre plots. One plot received four equal applications of nitrate of soda at a total rate of 400 pounds an acre; the other plot received one application at a rate of 100 pounds an acre. There was also a difference in the total fertilizer applied, the high-nitrogen plot receiving 849 pounds of an 8-4-3 and the low-nitrogen plot 196 pounds of the same mixture.

Marked responses to additions of nitrogen are obtained in field plots, since the soils of this region are very deficient in this element. The two treatments thus produced very appreciable differences in plant response as shown by general vigor, green color, height, and number of matured bolls per plant.

The gross features in plant response not only were affected, but the results given in Table 2 and Figures 4 and 5 seemingly indicate appreciable differences in toughness of the boll wall. A few exceptions to the general trend will be noted, yet these might be expected since a 10-boll sample is not considered a fully representative one. It is believed that the use of approximately 65 to 100 carefully performed

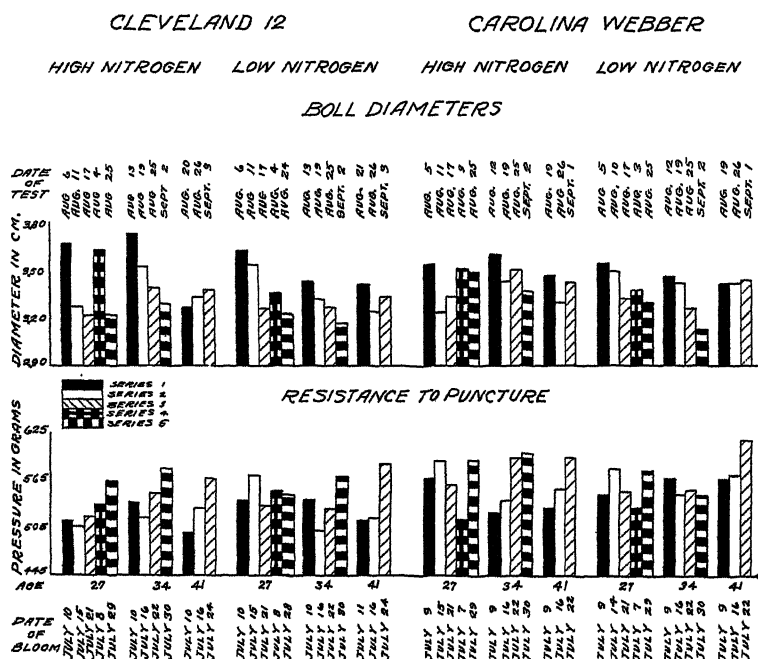


FIG. 4.—Diameter and resistance to puncture, Cleveland 12 and Carolina Webber cotton bolls, 1925

puncture tests on 10 bolls gives a fairly reliable index for the sample studied as shown by the standard error calculations,<sup>4</sup> but the sample was not always fully representative of the field, as indicated by boll diameter measurements which were also taken. Another factor that undoubtedly leads to variations, at least in the older bolls, is the difference in the rate of maturity, which has been previously mentioned. There are also differences in the earliness of varieties, Dixie Triumph being generally the latest variety of the three tested. Thus, it is not improbable that seasonal conditions may produce a state of succulency in a 41-day boll formed late in the season comparable to that of a 34-day boll formed early in the season.

<sup>4</sup> Standard error is used in preference to "probable error." For discussion, see Engledow, F. L., and Yule, G. Udny (4).



TABLE 2.—Results of an average puncture test upon 10 cotton bolls of 3 varieties at 27, 34, and 41 days of age, 1925

Series No.	High nitrogen						Low nitrogen					
	Date of bloom	Date of test	Wall thickness	Boll diameter	Age	Pressure <sup>a</sup>	Date of bloom	Date of test	Wall thickness	Boll diameter	Age	Pressure <sup>a</sup>
Cleveland 12							Cleveland 12					
			<i>Cm.</i>	<i>Cm.</i>	<i>Days</i>	<i>Grams</i>			<i>Cm.</i>	<i>Cm.</i>	<i>Days</i>	<i>Grams</i>
1-----	July 10	Aug. 6	0.20	3.67	27	513±4.4	July 10	Aug. 6	0.19	3.63	27	542
	do.	Aug. 13	.20	3.75	34	537±3.7	do.	Aug. 13	.17	3.45	34	543
	do.	Aug. 20	.16	3.29	41	500±5.8	July 11	Aug. 21	.16	3.41	41	518
	July 15	Aug. 11	.17	3.29	27	507	July 15	Aug. 11	.18	3.55	27	571
2-----	July 16	Aug. 19	.17	3.53	34	517	July 16	Aug. 19	.16	3.33	34	504
	do.	Aug. 26	.15	3.35	41	530	do.	Aug. 26	.15	3.25	41	520
	July 21	Aug. 17	.17	3.22	27	518	July 21	Aug. 17	.17	3.27	27	534
3-----	July 22	Aug. 25	.18	3.40	34	552	July 22	Aug. 25	.16	3.28	34	526
	July 24	Sept. 3	.15	3.39	41	567	July 24	Sept. 3	.15	3.35	41	587
Incom- plete.	July 8	Aug. 4	.22	3.64	27	535	July 8	Aug. 4	.18	3.37	27	551
	July 29	Aug. 25	.17	3.23	27	564	July 28	Aug. 24	.17	3.24	27	549
	July 30	Sept. 2	.18	3.30	34	582	July 30	Sept. 2	.16	3.17	34	570
Carolina Webber							Carolina Webber					
1-----	July 9	Aug. 5	0.22	3.56	27	567±3.8	July 9	Aug. 5	0.21	3.55	27	549
	do.	Aug. 12	.20	3.61	34	525±3.7	do.	Aug. 12	.20	3.48	34	570
	do.	Aug. 19	.19	3.47	41	529±4.6	do.	Aug. 19	.17	3.43	41	568
	July 15	Aug. 11	.22	3.24	27	590	July 14	Aug. 10	.21	3.50	27	584
2-----	July 16	Aug. 19	.19	3.44	34	541	July 16	Aug. 19	.19	3.44	34	547
	do.	Aug. 26	.18	3.30	41	556	do.	Aug. 26	.18	3.43	41	574
	July 21	Aug. 17	.20	3.35	27	561	July 21	Aug. 17	.21	3.34	27	552
3-----	July 22	Aug. 25	.19	3.50	34	595	July 22	Aug. 25	.18	3.27	34	554
	do.	Sept. 1	.18	3.43	41	595	do.	Sept. 1	.19	3.44	41	620
Incom- plete.	July 7	Aug. 3	.21	3.52	27	517	July 7	Aug. 3	.23	3.39	27	532
	July 29	Aug. 25	.22	3.50	27	592	July 29	Aug. 25	.22	3.30	27	581
	July 30	Sept. 2	.21	3.38	34	599	July 30	Sept. 2	.20	3.13	34	552
Dixie Triumph							Dixie Triumph					
1-----	July 10	Aug. 6	0.19	3.48	27	493±3.6	July 10	Aug. 6	0.20	3.51	27	554
	do.	Aug. 13	.20	3.65	34	514±4.8	do.	Aug. 13	.18	3.51	34	555
	do.	Aug. 20	.17	3.55	41	481±4.3	do.	Aug. 20	.16	3.44	41	527
	July 15	Aug. 11	.18	3.33	27	510	July 16	Aug. 12	.19	3.33	27	553
2-----	July 16	Aug. 19	.18	3.41	34	502	do.	Aug. 19	.18	3.32	34	538
	July 17	Aug. 27	.17	3.41	41	533	July 17	Aug. 27	.19	3.46	41	552
	July 21	Aug. 17	.18	3.38	27	571	July 21	Aug. 17	.18	3.29	27	518
3-----	July 23	Aug. 26	.18	3.32	34	526	July 23	Aug. 26	.16	3.13	34	526
	July 22	Sept. 1	.16	3.30	41	637	July 22	Sept. 1	.15	3.20	41	560
Incom- plete.	July 8	Aug. 4	.21	3.68	27	552	July 8	Aug. 4	.21	3.70	27	583
	July 28	Aug. 24	.17	3.32	27	518	July 28	Aug. 24	.17	3.13	27	528
	July 30	Sept. 2	.17	3.22	34	576	July 30	Sept. 2	.17	3.10	34	541

<sup>a</sup> Each figure for pressure represents an average of 62 to 98 punctures of innermost boll wall.<sup>b</sup> Standard error.

A nitrogen deficiency seems to produce tougher bolls on Dixie Triumph from blossoms formed to July 17, inclusive, with a shift to tougher bolls from the high-nitrogen plot from blossoms produced after the above date. It appears that the same general tendency exists in the Cleveland variety, though there are a few exceptions.

There are seven pairs of boll samples for comparison of the Dixie Triumph variety from blossoms formed to July 17, inclusive. Bolls grown under conditions of low nitrogen show the greatest resistance to puncture at 27, 34, and 41 days, while bolls formed July 21 to 30, inclusive, show the tendency for greater toughness under conditions of high-nitrogen supply, which is particularly true of the 34-day and 41-day bolls tested September 1 and 2. All bolls tested to

August 27, inclusive, with two exceptions, therefore, are tougher with a low supply of nitrogen, and those tested later are tougher with a high supply of nitrogen.

The five pairs of boll samples of Cleveland 12 tested before August 19 show tougher boll walls with a low nitrogen supply, while five of the pairs tested beginning on that date offer greater resistance to penetration with a high-nitrogen supply. The two exceptions are 41-day bolls.

It appears that bolls of Carolina Webber at 27 days of age are usually tougher with a high-nitrogen supply, regardless of the period of development or date of test, but as they approach the stage of maturity represented by 41 days, they are tougher in general under low-nitrogen conditions. The differences in the boll reactions of the three varieties should be noted because of the probable relations to plant metabolism. The relative rates of toughening of boll walls of Cleveland 12 and Dixie Triumph from plants with a low and a high nitrogen supply, respectively, were reversed about August 19 to 25, for the toughest bolls in general at 27, 34, or 41 days occur on low-nitrogen plants before these dates and in general on high-nitrogen plants after these dates. Such differences in toughness may be indicative of changes in the relative levels of plant metabolism under the two conditions, with shifts in the relative rates of supply, or proportion of substances supplied to bolls occurring about August 19 to 25.

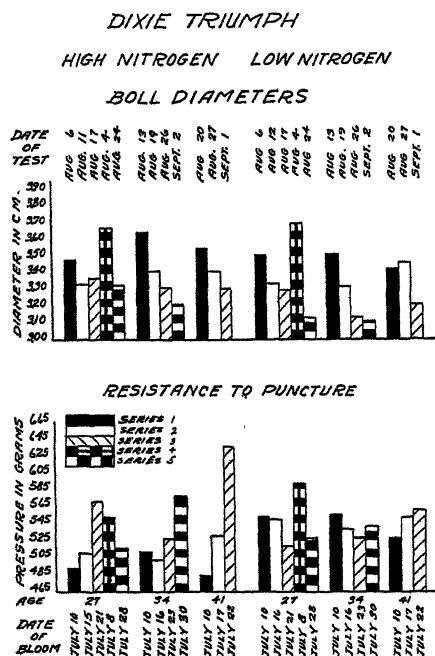


FIG. 5.—Diameter and resistance to puncture, Dixie Triumph cotton bolls, 1925

present as clearly the probable relations, but the fact that most 27-day bolls, both early and late, are toughest with high nitrogen and the 41-day bolls toughest with low nitrogen seems to indicate that changes in the rate of supply, or proportions of the substances supplied the boll, occur at regular stages in the development of the individual boll rather than at a definite stage of plant development, as in Cleveland 12 and Dixie Triumph.

The results presented show, therefore, that a pressure test on cotton bolls of the ages studied does detect certain differences which are due presumably to the conditions of fertility under which the plants were grown.

Regardless of conditions of fertility or the variety, however, there are certain tests in the series which depart considerably from the

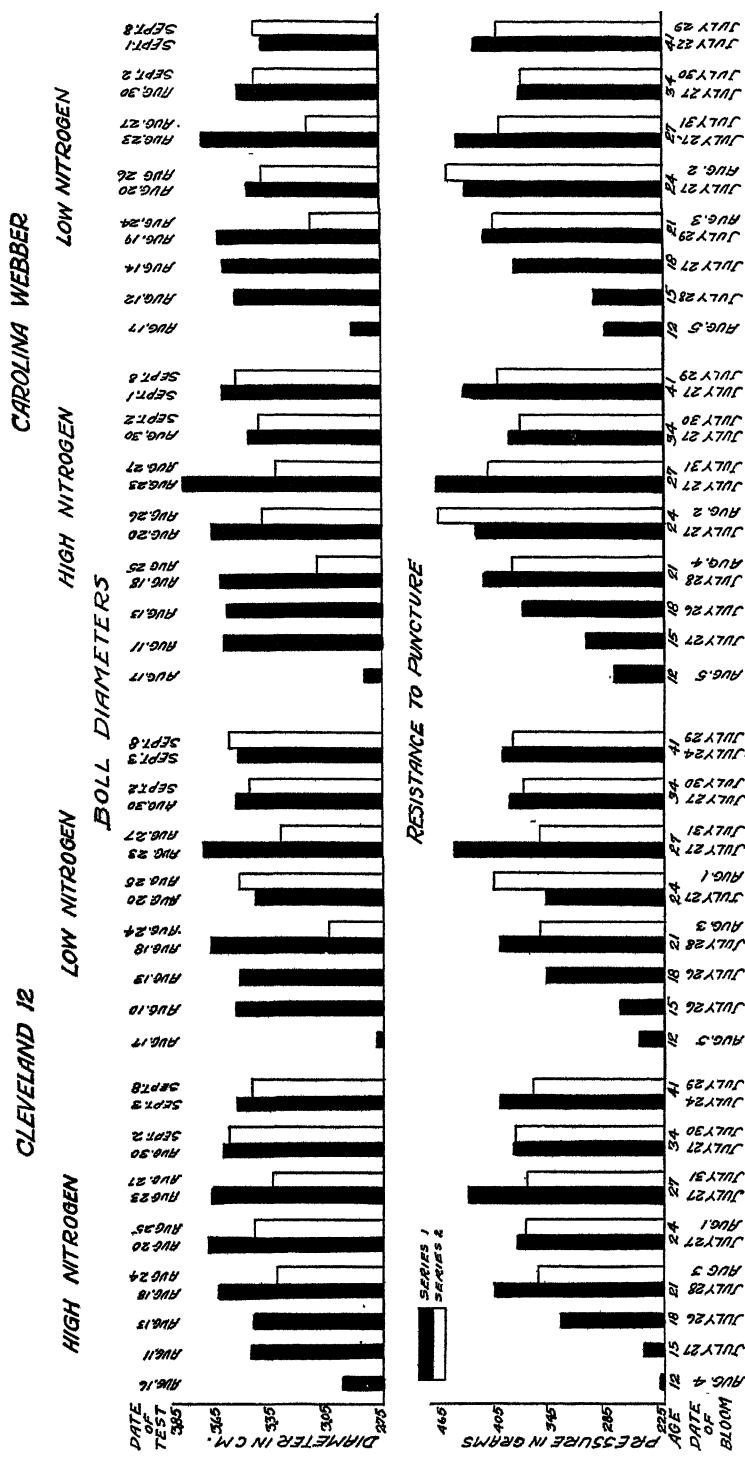


Fig. 6.—Diameter and resistance to puncture, Cleveland 12 and Carolina Webber cotton bolls, 1926

average, such as the relatively high pressures necessary to puncture most of the 34-day and 41-day bolls on September 1, 2, and 3. A few such exceptions occurred in 1924 and 1926. A study of records of temperature, humidity, rainfall, soil moisture, and evaporation from white and black atmometers inclines the author to believe that certain combinations of environmental conditions at the day of, and for two or three days preceding, the test are partly responsible,

though it has not been possible to evaluate these with any degree of certainty.

#### RESULTS OF PUNCTURE TESTS, 1926

The same varieties were used in 1926 as in 1925, and it was planned to have approximately the same conditions of high and low nitrogen,

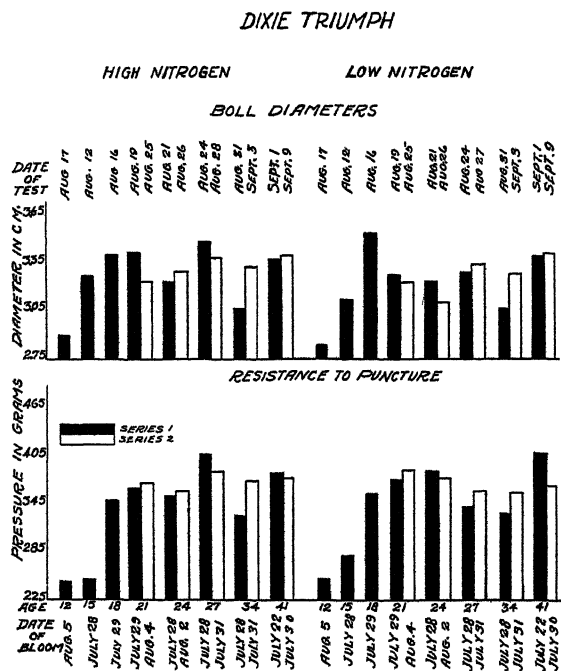


FIG. 7.—Diameter and resistance to puncture, Dixie Triumph cotton bolls, 1926

that the age of the boll is not a determining factor for toughness throughout its entire period of development. A daily series of boll-diameter measurements in 1927, which are not presented in this paper, show that the boll reached practically its full size in 20 days. Thus it seems that in general the period of boll development, characterized by rather regular increase in resistance to puncture, ceases at about full size, though this may occasionally continue to 41 days, as seen in certain instances throughout these tests.

It will be noted that bolls of the Webber variety were generally the toughest and that those of Dixie Triumph and Cleveland 12 assume variable relations to each other at almost every succeeding test.

but the plot selected proved to be so fertile that no appreciable difference in the plants could be detected until late in the season, even though the nitrate of soda was applied as in 1925. An examination of the data in Table 3 and Figures 6 and 7 shows that no general and significant differences are to be found in the toughness of bolls from high and low nitrogen plots.

There is a progressive increase in boll toughness with age in every series from 12 to 21 days, inclusive. This progressive increase does not occur from 24 days to 41 days, thus indicating

TABLE 3.—Results of an average puncture test upon 10 cotton bolls of 3 varieties at 12, 15, 18, 24, 27, 34, and 41 days of age, 1926

High nitrogen							Low nitrogen						
Series No.	Date of bloom	Date of test	Wall thickness	Boll diameter	Age	Pressure <sup>a</sup>	Date of bloom	Date of test	Wall thickness	Boll diameter	Age	Pressure <sup>a</sup>	
Cleveland 12							Cleveland 12						
1-----	Aug. 4	Aug. 16	<i>Cm.</i> 0.17	<i>Cm.</i> 2.96	<i>Days</i> 12	<i>Grams</i> 229±2.4	Aug. 5	Aug. 17	<i>Cm.</i> 0.17	<i>Cm.</i> 2.78	<i>Days</i> 12	<i>Grams</i> 249	
	July 27	Aug. 11	.19	3.47	15	249±3.2	July 26	Aug. 10	.21	3.55	15	272	
	July 26	Aug. 13	.19	3.46	18	338±4.2	do.	Aug. 13	.20	3.51	18	353	
	July 28	Aug. 18	.21	3.65	21	410±7.4	July 28	Aug. 18	.19	3.68	21	405	
	July 27	Aug. 20	.19	3.70	24	383±5.4	July 27	Aug. 20	.17	3.44	24	352	
	do.	Aug. 23	.22	3.68	27	438±6.0	do.	Aug. 23	.22	3.71	27	454	
2-----	do.	Aug. 30	.19	3.60	34	388±2.8	do.	Aug. 30	.18	3.54	34	392	
	July 24	Sept. 3	.18	3.55	41	405±4.4	July 24	Sept. 3	.17	3.53	41	401	
	Aug. 3	Aug. 24	.18	3.33	21	363	Aug. 3	Aug. 24	.17	3.05	21	362	
	Aug. 1	Aug. 25	.18	3.45	24	381	Aug. 1	Aug. 25	.19	3.52	24	412	
	July 31	Aug. 27	.18	3.35	27	373	July 31	Aug. 27	.18	3.30	27	362	
	July 30	Sept. 2	.20	3.59	34	387	July 30	Sept. 2	.20	3.47	34	377	
July 29	Sept. 8	.18	3.46	41	367	July 29	Sept. 8	.19	3.57	41	390		
Carolina Webber							Carolina Webber						
1-----	Aug. 5	Aug. 17	0.19	2.84	12	276±2.6	Aug. 5	Aug. 17	0.18	2.90	12	288	
	July 27	Aug. 11	.22	3.60	15	310±4.5	July 28	Aug. 12	.20	3.53	15	301	
	July 26	Aug. 13	.22	3.59	18	380±4.3	July 27	Aug. 14	.22	3.59	18	395	
	July 28	Aug. 18	.22	3.62	21	420±4.3	July 29	Aug. 19	.19	3.62	21	422	
	July 27	Aug. 20	.20	3.66	24	428±3.9	July 27	Aug. 20	.17	3.47	24	441	
	do.	Aug. 23	.23	3.81	27	469±4.8	do.	Aug. 23	.20	3.70	27	448	
2-----	do.	Aug. 30	.20	3.46	34	394±4.8	do.	Aug. 30	.17	3.50	34	380	
	July 22	Sept. 1	.20	3.61	41	443±5.7	July 22	Sept. 1	.18	3.39	41	431	
	Aug. 4	Aug. 25	.19	3.10	21	390	Aug. 3	Aug. 24	.19	3.13	21	411	
	Aug. 2	Aug. 26	.22	3.40	24	469	Aug. 2	Aug. 26	.20	3.39	24	457	
	July 31	Aug. 27	.21	3.32	27	413	July 31	Aug. 27	.19	3.24	27	402	
	July 30	Sept. 2	.20	3.41	34	382	July 30	Sept. 2	.19	3.43	34	379	
July 29	Sept. 8	.20	3.54	41	405	July 29	Sept. 8	.18	3.44	41	405		
Dixie Triumph							Dixie Triumph						
1-----	Aug. 5	Aug. 17	0.18	2.90	12	246±4.2	Aug. 5	Aug. 17	0.16	2.84	12	253	
	July 28	Aug. 12	.19	3.28	15	250±2.9	July 28	Aug. 12	.18	3.12	15	281	
	July 29	Aug. 16	.19	3.40	18	346±4.3	July 29	Aug. 16	.19	3.54	18	367	
	do.	Aug. 19	.19	3.42	21	361±3.8	do.	Aug. 19	.17	3.26	21	375	
	July 28	Aug. 21	.17	3.23	24	357±5.0	July 28	Aug. 21	.16	3.23	24	386	
	do.	Aug. 24	.19	3.47	27	407±5.0	do.	Aug. 24	.16	3.28	27	348	
2-----	do.	Aug. 31	.16	3.06	34	330±5.5	do.	Aug. 31	.14	3.06	34	334	
	July 22	Sept. 1	.18	3.37	41	385±5.6	July 22	Sept. 1	.18	3.39	41	407	
	Aug. 4	Aug. 25	.17	3.23	21	372	Aug. 4	Aug. 25	.17	3.22	21	388	
	Aug. 2	Aug. 26	.17	3.29	24	363	Aug. 2	Aug. 26	.16	3.10	24	379	
	July 31	Aug. 27	.19	3.38	27	386	July 31	Aug. 27	.18	3.33	27	364	
	do.	Sept. 3	.19	3.32	34	374	do.	Sept. 3	.18	3.28	34	361	
July 30	Sept. 9	.17	3.39	41	376	July 30	Sept. 9	.18	3.41	41	370		

\* Each figure for pressure represents an average of 62 to 98 punctures of innermost boll wall.

† Standard error.

## BOLL DIAMETERS

There was no apparent relation between boll size and toughness in 1924 or 1925, as seen in Figures 3, 4, and 5, but the similarity of the graphs for 1926, as seen in Figures 6 and 7, is quite evident. If the 24-day-old bolls tested on August 25 and 26, 1926, had not shown relatively greater toughness than others in the series, the resemblance of the graphs would be very striking.

The results of 1924 gave some indication that bolls of 27 or 34 days of age when formed later in the season are somewhat smaller than bolls of the same ages formed earlier. The 41-day bolls were rather

variable in 1924 as they were throughout the three seasons. Again in 1925 bolls at the ages of 27 and 34 days were generally smaller when formed later in the season. Bolls formed later in the season of 1926 showed the same general tendency to be smaller at 21, 24, 27, and 34-days of age. Martin, Ballard, and Simpson (13) have reported that later bolls of the Lone Star variety grown in Texas were consistently smaller than the early ones. This was ascribed to a drought which checked the growth of the plants after the first week in August.

That 41-day bolls do not show a more regular tendency to be smaller late in the season, aside from the variations to be expected from 10-boll samples, may be because some of the earlier samples were dried almost to the point of cracking open, while later samples were succulent. It is quite probable that such changes in the condition of succulency cause considerable variation in the diameter of older bolls, though the extent of these differences is not known. Even daily variations in the diameter of bolls are known to occur. In an experiment of 1927, to be published later, contractions of 0.01 to 0.11 centimeter occurred between 9 a. m. and 4.30 p. m. with bolls from 20 to 49 days of age. The evaporating power of the air was not at all excessive as shown by black and white atmometers, and the soil moisture was abundant. Many of the measurements in 1925 and 1926 were made at times of very hot weather and low soil moisture, which might cause greater variations in boll diameter. The data of Table 2 give indications of a possible shrinkage of bolls from plants grown with a low nitrogen supply under the dry conditions of 1925. A comparison of the diameters of the 27-day and 34-day bolls of the same blossoming date grown with a low supply of nitrogen shows that 10 of the 12 samples are smaller at 34 days. The season was very dry and by the time of the later tests the plants in both the low and high nitrogen plots were showing signs of great water deficiency during the hot hours of the day. The low-nitrogen plants suffered particularly as was shown by the fact that they changed from a bright green to a light yellow color and shed all the young fruit and quite a number of the lower leaves. The shrinkage indicated here, however, was greater than that measured in 1927.

#### BOLL-WALL THICKNESS

Immediately after the puncture tests of 1925 and 1926, each boll was cut transversely along the circumference of the punctures and two measurements of wall thickness made at opposite points. The measurements were made to hundredths of a centimeter with a caliper that could be read to that degree of accuracy. Each figure for wall thickness in Tables 2 and 3 is the average of a 10-boll sample, or an average of 20 such measurements.

The data presented for 1925 in Table 2 show no evident relation of wall thickness and toughness. The figures for wall thickness do not follow the general trend of those for toughness within the ordinary range, nor is there any unusual thickness of wall associated with the rather tough bolls found in several of the tests made on September 1, 2, and 3.

No pronounced correlation of boll diameter and wall thickness can be found, though such a relation is indicated at the ages of 27 and

34 days. The figures for Cleveland 12 and Dixie Triumph particularly show that, in general, the larger the boll the thicker the wall will be. Webber generally produces the largest bolls and thickest walls. although the relation between boll diameter and wall thickness seems more variable for this variety.

A decrease in wall thickness of the 41-day bolls generally occurs for all varieties, depending largely on the degree to which the final drying of the boll has proceeded.

Table 3 presents the data on wall thickness for 1926. The results for wall thickness show fairly close agreement with those for boll diameter and boll toughness at 21, 24, 27, and 34 days of age. The similarity of the results can be extended to 15 and 18 days if wall thickness and boll diameter only are considered.

The decrease in wall thickness that occurs in the later stages of boll development is indicated in most of the 41-day measurements. This is in agreement with the results of 1925.

#### RELATION OF BOLL SIZE, BOLL-WALL THICKNESS, AND TOUGHNESS OF BOLL TO COTTON LOSS FROM BOLL-WEEVIL ATTACK

In 1925 and 1926, while the experiments reported in this paper were in progress, independent studies were made by Dunnam (3) of boll-weevil damage to bolls of known ages on the same varieties used for the needle-puncture tests. Not only were the varieties the same but the seed was obtained from the same source. The plants were not grown on the same plots, however, nor were the same amounts of fertilizer applied. Regardless of these differences, mention of some of the results does not seem amiss.

The boll-weevil studies for 1925 have been reported by Dunnam (3) and the results for both years will soon appear.<sup>5</sup> Weevils were caged on 2,833 bolls in the weevil-damage experiments of Fenton and Dunnam,<sup>5</sup> while about 136,000 needle punctures were made on 1,510 bolls in 1925 and 1926. Fenton and Dunnam found that the resistance of a boll to weevil damage progressively increases with boll age. Two of the varieties suffered less than 10 per cent cotton loss from weevil attack after the bolls were 20 days old. A third variety was more susceptible, the weevil being able to cause more than 10 per cent damage until the bolls were 30 days old. The results obtained by the writer from the needle-puncture tests do not show a close correlation of either resistance to puncture, wall thickness, or boll size to percentage of cotton loss from weevil attack. It is realized that the boll weevil does not puncture the boll wall by mere pressure, yet the toughness of the inner wall may be of some value, for Fenton and Dunnam<sup>5</sup> point out that dissections of older bolls often show that the hole made by the weevil for the reception of the egg extends only to the inner membrane surrounding the cottonseed and lint in each locule.

It should also be kept in mind that the greatest weevil damage occurs during the first 20 to 30 days of boll growth, while needle-puncture tests were begun with 20-day bolls for a few varieties in 1924, with 27-day bolls in 1925, and with 12-day bolls in 1926.

<sup>5</sup> FENTON, F. A., and DUNNAM, E. W. THE BIOLOGY OF THE COTTON BOLL WEEVIL AT FLORENCE, S. C. [Unpublished manuscript.]

Bolls with the toughest walls, such as Cleveland 12 in 1924, suffered the least cotton loss until about 27 days in the early tests, while the bolls that remained longest in a comparatively tender state, as in the Dixie Triumph variety, suffered the greatest cotton loss. In the tests of the following years, the walls of Webber bolls have generally been the toughest and the thickest, although this variety has not suffered the lowest percentage of cotton loss. A larger number of records on bolls of individual ages are necessary before definite conclusions can be drawn.

#### SUMMARY

Cotton boll size, wall thickness, and toughness, as measured by resistance to needle puncture, have been studied on bolls of several ages from different varieties grown under two conditions of fertility. The tests were conducted during three seasons of growth.

There was an increase in toughness of boll wall to about 21 days, at which time the bolls had practically attained their full size. After this time the age of the boll was not a chief factor.

The conditions during the developmental period probably influence the relative toughness of walls, as shown by the generally more tender bolls in the later series of 1926 contrasted with the tougher bolls in some of the late series of 1924.

The conditions of fertility may be reflected in the resistance to puncture if the contrast in fertility conditions is sufficiently great.

Varietal differences in toughness of wall are evident though not constant. Bolls of Webber were toughest late in the season of 1924 and in general throughout the seasons of 1925 and 1926. The relative toughness of the bolls of Dixie Triumph and Cleveland 12 at different ages was variable.

The bolls of Webber are, in general, largest and the walls thickest.

No close correlation was found between boll size, wall thickness, or wall toughness and the percentage of cotton loss from boll-weevil attack as studied by Fenton and Dunnam.<sup>6</sup>

#### LITERATURE CITED

- (1) CULPEPPER, C. W., and MAGOON, C. A.  
1924. STUDIES UPON THE RELATIVE MERITS OF SWEET CORN VARIETIES FOR CANNING PURPOSES AND THE RELATION OF MATURITY OF CORN TO THE QUALITY OF THE CANNED PRODUCT. *Jour. Agr. Research* 28: 403-443, illus.
- (2) ——— and MAGOON, C. A.  
1927. A STUDY OF THE FACTORS DETERMINING QUALITY IN SWEET CORN. *Jour. Agr. Research* 34: 413-433, illus.
- (3) DUNNAM, E. W.  
1926. COTTON BOLL GROWTH IN RELATION TO BOLL WEEVIL INJURY. *Jour. Econ. Ent.* 19: 589-593.
- (4) ENGLEADOW, F. L., and YULE, G. U.  
1926. THE PRINCIPLES AND PRACTICE OF YIELD TRIALS—I. *Empire Cotton Growing Rev.* 3: 112-146.
- (5) HARTMAN, H.  
1924. STUDIES RELATING TO THE HARVESTING AND STORAGE OF APPLES AND PEARS. *Oreg. Agr. Expt. Sta. Bul.* 206, 32 p., illus.
- (6) HAWKINS, L. A., and HARVEY, R. B.  
1919. PHYSIOLOGICAL STUDY OF THE PARASITISM OF *PHYTHIUM DEBARYANUM* HESSE ON THE POTATO TUBER. *Jour. Agr. Research* 18: 275-298, illus.

<sup>6</sup> FENTON, F. A., and DUNNAM, E. W. Op. cit.



- (7) HAWKINS, L. A., and SANDO, C. E.  
1920. EFFECT OF TEMPERATURE ON THE RESISTANCE TO WOUNDING OF CERTAIN SMALL FRUITS AND CHERRIES. U. S. Dept. Agr. Bul. 830, 6 p., illus.
- (8) LEWIS, C. I., MURNEEK, A. E., and CATE, C. C.  
1919. PEAR HARVESTING AND STORAGE INVESTIGATIONS IN ROGUE RIVER VALLEY. (SECOND REPORT.) Oreg. Agr. Expt. Sta. Bul. 162, 39 p., illus.
- (9) MAGNESS, J. R., and BURROUGHS, A. M.  
[1923]. STUDIES IN APPLE STORAGE. *In* Storage Investigations, 1921-1922. Marble Lab. Inc. Rpt. 2: 17-98, illus. Canton, Penn.
- (10) ——— and DIEHL, H. C.  
1924. PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE. Jour. Agr. Research 27: 1-38, illus.
- (11) ——— and TAYLOR, G. F.  
1925. AN IMPROVED TYPE OF PRESSURE TESTER FOR THE DETERMINATION OF FRUIT MATURITY. U. S. Dept. Agr. Circ. 350, 8 p., illus.
- (12) MAGOON, C. A., and CULPEPPER, C. W.  
1926. THE RELATION OF SEASONAL FACTORS TO QUALITY IN SWEET CORN. Jour. Agr. Research 33: 1043-1072, illus.
- (13) MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M.  
1923. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 195-208, illus.
- (14) MORRIS, O. M.  
1925. STUDIES IN APPLE STORAGE. Wash. Agr. Expt. Sta. Bul. 193, 44 p., illus.
- (15) MURNEEK, A. E.  
1921. A NEW TEST FOR MATURITY OF THE PEAR. PEAR HARVESTING AND STORAGE INVESTIGATIONS. (THIRD REPORT.) Oreg. Agr. Expt. Sta. Bul. 186, 28 p., illus.
- (16) NELLER, J. R., and OVERLEY, F. L.  
1926. PHYSICAL AND CHEMICAL CHARACTERISTICS OF MATURING APPLES AS RELATED TO TIME OF HARVEST. Wash. Agr. Expt. Sta. Bul. 205, 38 p., illus.
- (17) ROSENBAUM, J., and SANDO, C. E.  
1920. CORRELATION BETWEEN SIZE OF THE FRUIT AND THE RESISTANCE OF THE TOMATO SKIN TO PUNCTURE AND ITS RELATION TO INFECTION WITH MACROSPORIUM TOMATO COOKE. Amer. Jour. Bot. 7: 78-82.
- (18) RUDNICK, R. A., and BAKKE, A. L.  
1920. THE MECHANICAL PENETRATION OF THE SWEET CORN PERICARP. Iowa Acad. Sci. Proc. 34: [129]-132, illus.
- (19) WILLAMAN, J. J., PERVIER, N. C., and TRIEBOLD, H. O.  
1925. BIOCHEMISTRY OF PLANT DISEASES V. RELATION BETWEEN SUSCEPTIBILITY TO BROWN ROT IN PLUMS AND PHYSICAL AND CHEMICAL PROPERTIES. Bot. Gaz. 80: 121-144, illus.



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